Parietal cortex and spatial cognition : combining evidence from neuroimaging and functional brain stimulation

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Kijk om je heen, kijk om je heen
Zoals de wereld nu is
Zie je hem nooit weer

C. Buddingh

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Chapter 1

General introduction

In each human being houses approximately one and a half kilograms of utter mystery: the brain. Despite its undisputed significance with regard to almost every physical and mental process, and despite all the things that can – and do – go wrong with it, staggeringly little is known about its functional architecture. The brain mainly consists of nerve cells or *neurons* – approximately a hundred billion of them - which constitute the so-called grey matter or *cortex*, and the fibres connecting these cells, the so-called white matter, the electrical wiring transporting the small electrical signals or *action potentials* which are produced by a neuron to convey a message to one or several other neurons. Each neuron is connected to between one thousand and ten thousand other neurons. One can think of the brain as a giant switchboard, connecting thousands, millions of calls in parallel, but at the same time managing to extract a distinct set of general, meaningful conclusions from this cacophony of messages, leading to the manifestation of certain states, thoughts or actions. And, importantly: there is no central control. To the best of current knowledge, no single entity or process oversees all this activity. Yet, these billions of individual cells manage to work together in an organised manner. Of course, there is rivalry among them. Connections which are not used waste away, and brain areas which do not receive or send input on a regular basis are invaded by neighbouring areas which are always trying to expand their territory. Yet, most of the time, the brain acquits itself of its tasks with the precision of a Swiss clockwork, without disclosing anything to its owner about the continuous stream of informational processes underlying its functioning. It is usually not until something gets stuck between the clock's cogwheels, that it becomes clear precisely how flawlessly many parallel functions are normally combined by the brain. It is only then that repairing the clockwork becomes crucial, so that it can return to its normal functioning. However, in order to be able to repair something, it is paramount to first know how it works. The field of cognitive neuroscience aims at exactly this: unravelling the functional architecture of the normally functioning brain, revealing how it is that billions of individual cells work together forming a functional unit, and how it is that such units cooperate within a network, merely by sharing and exchanging information in the form of tiny electrical potentials travelling along connecting fibres.

Each chapter of this thesis represents an attempt to identify a piece of this puzzle, and to assess how these pieces relate to each other. Different methodological approaches were explored, and their advantages, pitfalls and interrelations are evaluated – always in light of the general main goal of identifying the functional architecture of the normally functioning brain, in order to eventually enable us to fix broken clockworks.

For a long time, scientists interested in the relationship between brain architecture and behavioural performance did not have much choice but to behaviourally observe patients who, after serious head injury or other possibly brain-related misfortunes, displayed abnormal behaviour or loss of function. After the patient deceased, the brain could be inspected post-mortem, and any visible changes could then post hoc be related to changes in behaviour during life. Using this approach, much of the coarse functional layout of the brain could be mapped. However, there are several limitations with regard to studying the functional architecture of the brain relying on the cognitive and behavioural consequences of brain malfunctions. Damage or 'lesions' to the brain caused for example by a stroke are often massive anatomical disruptions, stretching over several square centimetres of cortical surface. Considering that one square centimetre of cortex contains approximately 40 million neurons, any lesion will inevitably envelope several - possibly distinct - functional units. Hence, lesions are rarely small enough to be confined to a single functional area or module in the brain, which means that their resulting deficiencies often pervade into multiple functional domains. This makes it difficult to ascribe certain functions to specific parts of the brain. Secondly, the cognitive consequences of a lesion are not stable over time. Especially in the first days and weeks following lesion onset, the brain goes through intense processes of plastic changes, functionally reorganising itself, trying to compensate for the damage and maintain as much functionality as possible. As a result of this cortical plasticity, the functional layout of the brain is changed, which could lead to an underestimation (or even overestimation in case of maladaptive plasticity) of the relevance of a certain brain area for a given function in the healthy brain (Sack, 2010). Thirdly, no two lesions are exactly the same with regard to their location, size and functional impact, which further distorts comparisons between lesions and their functional consequences. Fourthly, patients suffering from lesion-induced loss of brain function are in the majority of the cases only monitored from the moment they report their deficits and seek treatment. This makes it impossible to objectively compare cognitive brain functioning after the lesion to cognitive functioning in the same person before lesion occurrence, when the brain was presumably still healthy. Finally, in the same line of thought, differences in age, education, personality, and other possibly confounding background factors cannot be controlled for, for the simple reason that patients can by definition not be randomly assigned to a patient or non-patient group.

Despite these limitations, studying patients suffering from altered cognitive functioning as a result of brain damage has so far been an extremely important source of information about the functional architecture of the healthy brain. Some of the limitations mentioned above can partially be counteracted by taking

measures such as investigating only patients in the acute state of a lesion, and by comparing patient characteristics to those of healthy controls, which are preferably matched to the patient group with regard to most confounding criteria such as age and educational level. Nevertheless, technical progress has provided us with more elegant and efficient ways to investigate the relationship between brain and behaviour under controlled experimental conditions, and to study brain functions of neurologically healthy, randomly selected adults who can serve as their own controls when comparing behavioural performance with and without local neural activity disruptions.

Since brain imaging and brain interference techniques started to develop during the nineteen eighties, cognitive neuroscience has made, and still continues to make, tremendous progress in the quest for unravelling the neuronal mechanisms underlying cognitive functioning. The work presented in the subsequent chapters has mainly focussed on how one of the most fascinating and underexplored parts of the brain, parietal cortex, enables the highly complex cognitive process of spatial cognition, thereby employing different combinations of three cognitive neuroscience methods: functional magnetic resonance imaging (fMRI), electro-encephalography (EEG), and transcranial magnetic stimulation (TMS). In order to first create a general understanding of why and how these methods were employed and combined, in the following section each method will be described, and their advantages and disadvantages will be outlined. Subsequently, the anatomical and functional properties of parietal cortex and its relation to spatial cognition will be discussed.

Functional Magnetic Resonance Imaging (fMRI)

First, it is important to distinguish between MRI and fMRI. Magnetic resonance imaging (MRI) allows for visualisation of anatomical structures, including the brain. Thinking back to the previously described practice of post-mortem inspection of the damaged brain of a patient who during life displayed abnormal behaviour, it is obvious that a method which allows for *in vivo* imaging of the lesioned brain has the potential to greatly improve the quality of life of the patient, as well as that of the scientist. Along with other structural imaging methods like computer tomography (CT), MRI became common property during the nineteen eighties. In contrast to CT scans, which involve X-rays and intravenously administered contrast agents, MRI scans are very safe and completely non-invasive, and provide structural images of the brain in great detail.

While the use of structural MRI mainly provided great improvement for the clinical practice, the quest for mapping the functional layout of the brain really leaped forward after functional MRI (fMRI) was developed. Two discoveries were paramount for the development of fMRI. Already in 1890, Roy and Sherrington (Roy & Sherrington, 1890) described how hemodynamic variations in the brain are modulated by ongoing neural activity. Hence, if a neuron becomes more active, it consumes more oxygen, which is subsequently replenished by an increased supply of oxygenated blood to the location of the active neuron. The second milestone took place one hundred years later, when Ogawa and colleagues (Ogawa, Lee, Kay, & Tank, 1990) presented evidence that deoxyhemoglobin, which is naturally present in blood, acts as a contrast agent for MR imaging. Using gradient-echo recording sequences, they were able to visualise changes in the blood oxygen level in the MR image. They suggested that this blood oxygenation level-dependent (BOLD) contrast could be used to provide in vivo real-time maps of blood oxygenation in the brain during functional processing.

Indeed, the BOLD-signal proved an extremely useful asset for brain imaging. During the nineties, a rapidly increasing number of publications based on fMRI recordings were published. By contrasting the activation pattern observed during the execution of a certain task, for example observing a visual stimulus, speaking, or pressing a button, with that observed during rest, a pattern representing specifically the brain areas involved during this task could be visualised. Using this approach, and employing increasingly complex tasks, much of the coarse functional architecture of the brain could be mapped. Compared to the most frequent other functional brain imaging method, positron-emission tomography (PET), fMRI has the advantage that it does not require any injection of radioactive tracers, it is a non-invasive and safe method. The spatial resolution of an fMRI image is expressed by the size of one *voxel*, which is a threedimensional unit comparable to the twodimensional pixel unit used in digital photography. Functional images are usually acquired with a voxel size of around 3 mm^3, although smaller voxel sizes approaching 1 mm^3 are now also within range.

Contrasting task-related activation to baseline is the most common and straightforward approach to identify individual brain regions more or less co-activating with task demands, an approach which works especially well with relatively low-level processes. However, it has become increasingly clear that it is an oversimplification to assume that highly complex cognitive functions are processed within single, spatially confined brain regions. Rather, they are processed within widely distributed networks displaying a certain network connectivity and certain network dynamics. Hence, in addition to the simple contrast approach, numerous other procedures have been developed to extract

more finegrained information about functional activation from the timecourse of fMRI recording. Many of these procedures aim to visualise the networks of activation related to task processing, such as independent component analysis (ICA) which identifies spatially related activation clusters from a bottom-up perspective, or Granger causality mapping which visualises effective connectivity between brain areas in a network. Depending on the research question and the employed design, there are many alternatives beyond contrasting task-related activation to baseline, which are able to provide the level of detail of information that investigating highly complex cognitive tasks requires.

fMRI provides cognitive neuroscientists with the possibility to visualise ongoing brain activity in vivo, and moreover, during functional task execution. It allows scientists to map which brain areas are involved in which tasks, which functional networks exist in the healthy brain, if certain tasks rely on similar or different underlying mechanisms; the possibilities are endless. Advances with regard to the technical specifications, such as MRI scanners with stronger magnetic fields or better head coils, as well as ongoing developments in the analysis department, such as brain connectivity analyses, keep expanding the possibilities for further exploring the functional architecture of the brain. However, as the processes which are under investigation become more complex and detailed, it becomes increasingly important to consider, and accurately deal with, the shortcomings associated with fMRI. These shortcomings can be more or less problematic depending on the research question and the design of a study.

Limitations of fMRI

The notion that fMRI relies on visualising changes in oxygenation in the blood supplied to different areas of the brain, implies several drawbacks. Firstly, it means that the functional image we are observing is no direct transcript of the activation patterns of neurons. Instead, it is an indirect measure, reflecting a local increase in oxygen demand which logically results from increased neuronal activity. Unfortunately it is still not clear exactly which neuronal activity this is. Intracortical recordings from monkey cortex allow scientists to record which neurons fire under which circumstances, providing extremely detailed temporal and spatial information about the functional dedication of certain neuronal populations. After combining fMRI and concurrent intracortical recordings from monkey cortex, Logothetis and colleagues (Logothetis, Pauls, Augath et al., 2001a) concluded that fMRI mainly reflects input and intracortical processes – including subtreshold activity - of an activated brain area, rather than the spiking output of local neurons. In other words, a brain area appearing as more active in an fMRI image

might not always represent excitation, but could also reflect an area receiving more input, or imposing inhibitive influence on a different brain area. In addition, while the neuronal output as documented by intracortical recording can be considered to sum up in a linear manner, this principle does not entirely hold for the BOLD-signal variations observed in fMRI. A region which is twice as active does not typically produce a BOLD-signal which is twice as high. This notion is important considering that most fMRI designs rely on the aforementioned contrast between activation patterns during execution of certain tasks, versus patterns acquired during rest or a different control condition. Baseline activation is subtracted from task activation to obtain information about the brain areas recruited specifically during this task, based on the assumption that the two sum up more or less linearly. Hence, in addition to using the simple contrast approach, it might in some cases be valuable to introduce multiple parametrically varying levels of task execution, which are expected to result in parametrically varying BOLD-responses in specific areas of the brain concerned with this part of the task. In other situations, using additional processing such as functional connectivity analysis might provide important additional information. In any case, the aforementioned observations warrant caution when interpreting fMRI data, and argue for well thought-out experimental designs.

A second drawback resulting from the fact that fMRI visualises hemodynamic processes in the brain, is that the increased supply of oxygenated blood after initial depletion takes on average 4 to 5 seconds to emerge. This reduces the temporal resolution of fMRI recordings to a similar scale. By comparing the timing of variations like onset or peak of the BOLD-signal between different brain areas, some conclusions can be drawn about the order of activation within a certain network. However, since onset and peak latencies of the BOLD signal can also naturally vary between different brain areas, this too should be approached with caution. Furthermore, the spatial resolution of fMRI is limited by the fact that it visualises metabolic changes in the brain. Considering that one square centimetre of cortex contains forty million neurons, a typical functional voxel of 3 mm^3 still holds about four million neurons. Which are all connected to as much as ten thousand other neurons. Consequently, the signal measured from one voxel of the brain reflects the averaged intracortical signals of millions of neurons – possibly with heterogeneous functions.

A third major limitation of fMRI research is that it provides merely observational data about the relationship between behavioural or cognitive processes on the one hand, and brain activation patterns on the other. We can state that the activation level of a given area of the brain does or does not correlate with behavioural performance, but we cannot conclude that the

functioning of this brain area thus constitutes a prerequisite for behavioural performance. Carefully designing the tasks and conditions employed during the fMRI experiment allows us to narrow down the putative functions of a certain brain area as much as possible. However, the fact remains that causal attributions about an observed brain-behaviour association cannot be made purely on the basis of fMRI data. Additional methods are needed to answer these questions, such as functional brain interference techniques.

In addition to these three major drawbacks of fMRI as a method for investigating the neural correlates of behavioural and cognitive functioning, there are several less severe issues concerning this method, each of which can however prove to be a serious obstacle for studies focussing on certain research questions. MRI scanning as a whole, but especially functional MRI scanning, is an extremely noisy process, resulting in acoustic noise levels exceeding 120 dB. Although participants are always protected by wearing earplugs and headphones, they still receive a considerable amount of acoustic stimulation, resulting in high baseline activation of auditory processing areas in the brain, and possibly obscuring details of an auditory stimulus. This is especially problematic for studies aiming to investigate neural correlates of auditory or speech processing. The non-linearity of the BOLD-signals becomes more evident as baseline activation increases, until finally even reaching an activation ceiling. Hence, the loudness of the fMRI acquisition itself severely hampers the investigation of finegrained acoustic processing in the brain. There are ways around this issue, such as the sparse temporal acquisition (STA) recording scheme, which smartly exploits the temporal delay of the BOLD-signal by presenting auditory stimuli during a silent gap and acquiring fMRI data directly afterwards, thereby separating cortical activation related to the experimental auditory stimulus from that caused by the scanner noise (Amaro, Williams, Shergill et al., 2002).

A second minor consideration for fMRI acquisition is that, very much like during having a picture taken, the subject should move as little as possible. Head movement while functional or anatomical data are being collected results in worse quality of data, and movement in between recording spells creates issues later on during the analysis phase, when functional and anatomical data have to be co-registered in space. Considering that an fMRI session usually takes one to one and a half hours to complete, it can be a challenge to fulfil this requirement. But the true consequences of this drawback again, like in the case of scanner noise, depend on the research question of the study. If a study aims to investigate the neural correlates of motor processes, or motor-related processes such as motor mental imagery or the mirror neuron system (see Chapter 2), it faces a considerable restriction concerning the type and amount of movement which is

possible, both in terms of the very limited space available in the MRI scanner, as well as with regard to creating movement artefacts in the data. Presupposing that the head should remain as still as possible, few possibilities beyond finger movements remain. Even if the head is fixated with cushions, as is usually the case during fMRI acquisition, movement of the extremities will inevitably result in head movements. This clearly limits the opportunities for (re)creating natural and ecologically valid motor actions in the scanning environment.

In addition to being extremely noisy, and providing limited possibilities for movement, fMRI scanning is also a time-consuming and especially an expensive affair. This factor might be more relevant in countries with limited financial resources for scientific research, but it might become more relevant overall, as research budgets shrink. The high costs make it unattractive to test large populations of a hundred or more participants, as is for example common in behavioural, psychological and clinical studies, and could be considered a prerequisite for generalising findings across the general population. Fortunately, fMRI provides a very sensitive measure of brain functioning, and by comparing within-participant effects of different carefully designed tasks or stimuli, many irrelevant sources of variation can be eliminated. Using random-effects statistics further warrants extrapolation of results across the general population.

In summary, over the past twenty years fMRI has proven an increasingly powerful tool in the quest for unravelling the functional architecture of the healthy brain. In addition, it is increasingly being used for visualising the functional aberrations of the abnormally functioning brain, and even clinical applications are starting to emerge (Sorger, Dahmen, Reithler et al., 2009). However, fMRI has several limitations, some of which are inevitable and at the least warrant caution concerning the interpretations of results, and some of which can be overcome by careful experimental design and analysis. In any case, the advantages and opportunities offered by fMRI, especially in terms of spatial resolution, are not parallelled by any alternative method, and continuing technical and methodological developments will only increase its impact as a functional imaging tool.

Electro-encephalography (EEG)

Another method frequently used in the process of investigating the neural correlates of human cognition and behaviour is electro-encephalography (EEG). As previously described, neurons communicate with each other by transmitting small electrical signals, action potentials, along the axons which interconnect them.

While intracortical recording has the potential to directly measure these neuronal outputs, it requires surgical opening of the skull and insertion of a recording probe directly into the cortex, which is why using this invasive method is only allowed in animals and not in humans. However, the potentials created by the summed activity of billions of neurons eventually emerge through the skull, and can thus be recorded by electrodes placed directly on the scalp. Hence, EEG refers to the continuous extracranial recording of the summed brain potentials emitted by active neurons in the cortex. The signals reflected in these continuous recordings are osscilating in different frequency bands, ranging from delta (less than 4 Hz) to gamma bands (up to 80 Hz). Different bands reflect different underlying processes, and aberrant signals can reveal abnormal brain functioning. Consequently, continuous EEG recording is used frequently in the clinical practice, but it does not reveal much about the functional architecture of the healthy brain.

In addition, there is a different approach to using the electrical signals recorded from the scalp. Superimposed on the continuous waves recorded by the different electrodes are small signal variations which are linked to the occurrence of cognitive or behavioural events, such as seeing a visual stimulus, or hearing a sentence. Each signal increase or decrease by itself is almost indetectably small, but by overlaying and averaging fragments of the continuous signal which are time-locked to the stimulus event, the continuous signal and random noise are cancelled out, and only event-related fluctuations remain. This approach of processing EEG data is called event-related averaging (ERP). Event-related averages (ERPs) show in great temporal detail the electrical fluctuation of the brain in response to certain tasks or stimuli. This constitutes one of the biggest selling points of ERP: it has the superb temporal resolution of 1 ms, especially compared to the 5 second resolution of fMRI. Information about the temporal onset or the order of different processes can be obtained by contrasting different conditions with each other or with baseline. ERP is also a non-invasive, safe method to use, and compared to fMRI it is relatively cheap and simple to set up and use.

Limitations of EEG/ERP

Despite these benefits, ERP also has several inherent drawbacks, which are important to consider in light of data processing and interpretation. Firstly, it has a low spatial resolution. Although separate ERPs can be obtained from each recording electrode, which can be as much as 128, this does not mean that the signal recorded from a certain electrode is produced by neuronal activity directly underlying this electrode. Unlike magnetic signals which can pass through the skull unhampered, electrical signals are conducted differently by different tissues, such as grey or white matter, bone, muscle and skin. This results in the so-called

inverse problem of ERP localisation. On their way from the neurons to the scalp, the brain potentials are distorted unevenly by different tissues they come across, eventually emerging from possibly a different spatial recording site than the one directly overlying the neuronal population which they emerged from in the first place. Hence, it is difficult to localise the spatial source of an observed ERP effect with much more precision than the lobe it was recorded from. A possible solution for this problem is dipole modelling, in which a computational model of the head and the different tissues is used to retrace the most likely spatial origin of a signal. However, this approach demands that certain recording requirements are met, and is not a straightforward procedure. A second solution to the inverse problem of ERP localisation is to combine ERP with fMRI recordings, thus taking advantage of the high spatial resolution. Although possible, also this solution constitutes a methodological challenge.

Another, less problematic drawback, which should however be considered when designing the setup and when choosing the recording and analysis settings, is that the EEG and consequently the derived ERP signal are very weak in comparison to numerous omnipresent sources of electrical noise, such as electrical power cables. As a result, the recorded signal needs to be amplified as early as possible in the recording circuit, and care should be taken to avoid sources of electrical noise in the recording environment. In addition, the weak ERP signal is very sensitive to movement artefacts, which induce noise. Even eye blinking results in problematic signal distortions, which is why electro-oculograms (EOGs) should always be recorded along with the EEG/ERP recording, in order to enable post-hoc removal of trials which are contaminated by eye movements. It goes without saying that, like during fMRI acquisition, possibilities for studying motor processes are again severely limited. However, several research groups are working on EEG recording systems which would allow for bodily movement, by recording information about these movements and counteracting their disturbing influence on the EEG signal.

In summary, ERP is particularly suitable for unravelling the temporal characteristics of underlying cognitive processes, but detailed information about the spatial origin of these signals is not easily detectable.

Transcranial Magnetic Stimulation (TMS)

Whereas the previously described methods of fMRI and EEG/ERP are brain imaging or brain recording techniques, aiming at visualising ongoing cortical processing,

transcranial magnetic stimulation (TMS) is a brain interference technique. It relies both on the principle of electromagnetic induction and on the fact that neurons use electrical signals to communicate with each other, and combines these givens in order to temporarily and locally disrupt neuronal processing.

The principle of electromagnetic induction, which was first described by Faraday in 1831, states that an electrical current passing through a coil of metal wire induces a magnetic field, and the other way round, a magnetic field passing a wire coil induces an electrical current in this wire. A TMS machine basically consists of a battery of capacitators, which are able to generate high discharge electrical currents. These currents are sent through a magnetic coil, producing a magnetic field emerging from the surface of the coil. If this surface is placed on the scalp, the magnetic field passes through the scalp and enters the brain. Unlike electrical currents, which are unevenly distorted when passing through different kinds of tissue, a magnetic field is not distorted when passing though tissue, which means that the magnetic field enters the brain exactly under the magnetic coil. In the cortex, the axons which interconnect the neurons in the grey matter now serve as electricity conducting wires, allowing the magnetic field to generate small electrical currents in all the axons within its range. Hence, a TMS pulse causes excitatory currents similar to naturally occurring action potentials in the cortical area directly beneath the stimulation coil. Still, the net effect of this excitatory activation is a local and temporal loss of neuronal functioning. Considering the normally occurring action potentials of targeted neurons as signals used to speak to each other at a moderate level, the effect of TMS might best be compared to simultaneously making millions of neurons scream out loud. Hence, although TMS induces an electrical current very similar to a naturally occurring action potential, the fact that millions of neurons are excited at the same moment results in the loss of all finegrained communication signals between them.

As a consequence, compared to functional imaging which provides observational measures of the functional architecture of the healthy or diseased brain, TMS allows for direct local interference of cortical processing in a certain brain area, thus temporarily creating a 'virtual lesion' in conscious healthy volunteers. Observation of resulting behavioural changes provides causal information about the functional relevance of a certain brain area. If disruption of an area leads to impaired behavioural performance on a certain task, it is safe to conclude that this specific area plays a crucial role in executing this specific task. By contrasting different tasks, control conditions and stimulation sites, the specificity of such conclusions can be narrowed down further. By varying the intensity with which the TMS pulses are applied, also the effect TMS has on cortical functioning can be varied.

With this virtual-lesion approach of TMS, healthy volunteers can temporarily be turned into neurological patients, and back into healthy adults, under controlled experimental conditions (Sack, 2010). This allows for random selection of 'patients', or balanced selection with regard to a certain trade, if relevant. It also allows for within-person balanced comparison between behaviour observed with and without the virtual lesion, ruling out the many confounding factors that limit most patient studies. And whereas patients willing and able to participate in a scientific study might not always be easy to find, there is never a shortage of healthy volunteers.

Besides the application of TMS for cognitive neuroscience research aiming at exposing the functional layout of the healthy brain, it has also been used for many years as a clinical tool. It is known that several clinical disorders, such as depression, are associated with – or maybe even caused by - abnormal brain activity. TMS allows for treatment of over- or underactivated brain areas by de- or increasing local cortical excitability, allowing balance to return, and possibly creating a better mind set for receiving pharmacological and cognitive treatment. Although the clinical application of TMS is still in its infancy, it is already being used around the world as a valuable treatment option. As neural correlates of more disorders will be revealed, the potential of TMS as a therapeutic tool will only increase.

Different TMS application protocols

Depending on the research question and the circumstances of each study, different TMS protocols might be appropriate. TMS protocols might roughly be divided into two categories: event-related TMS (erTMS) and repetitive TMS (rTMS).

Event-related TMS refers to protocols in which TMS pulses are applied during individual experimental trials, and moreover, time-locked to certain task events, for example the onset of a visual stimulus. The number of pulses administered during each trial can vary, for example single, double or triple pulse TMS. The choice of how many pulses to apply each trial is made by comparative assessment. If there is no specific a priori hypothesis about the temporal window in which a certain target site is involved in task execution, and if it is also not the gist of the study to determine this, the safer choice might be to apply several pulses spread over a certain period of time, to increase the chance of disturbing ongoing processing. Not only is the area disturbed at multiple distinct timepoints, but in addition, several pulses administered in a sequence sum up to create a larger neuronal effect. If, in contrast, the aim of the study is to identify the temporal window of relevance and/or, in case of multiple stimulation sites, the order of recruitment of these areas, it is advisable to limit the number of pulses to

retain as much temporal specificity as is considered appropriate. By using this approach over several stimulation sites which are, for example, part of a functional network previously detected using fMRI, TMS can be used to systematically probe the temporal windows in which each area is engaged in task-related activities. Hence, employed in this manner, TMS becomes a tool for causally exploring functional connectivity within a complex cortical network.

Repetitive TMS refers to protocols in which typically several hundreds of pulses are administered over a continuous period of several minutes, at a certain stimulation frequency. On the account of the additive effects of TMS pulses administered in a sequence, rTMS has an effectivity which outlasts the actual stimulation period. In classical rTMS protocols, a single stimulation frequency is employed over the course of several minutes, for example 10 minutes of stimulation at a 1 Hz frequency, adding up to 600 pulses. Such protocols typically outlast the stimulation period by the same duration, thus after 10 minutes of stimulation the after-effect lasts for approximately 10 minutes as well. The stimulation frequency is paramount in such protocols: frequencies of 1 Hz or lower are known to locally suppress cortical processing, whereas frequencies above 1 Hz are known to increase cortical processing. However, since the effects of excitatory TMS on higher cortical areas are still elusive, the inhibitory rTMS protocol is much more commonly used than its excitatory counterpart.

Besides classical rTMS protocols which employ a single stimulation frequency for several minutes, in recent years several so-called patterned TMS protocols have emerged. Especially the theta burst stimulation (TBS) protocol, first described by Huang and colleagues (Huang, Edwards, Rounis et al., 2005), has quickly gained ground. The TBS protocol combines a very short application period with very long after effects, which make it very attractive. For only 40 seconds, three pulses are administered at 50 Hz, with these pulse trains recurring every 200 ms at 5 Hz. After stimulation has finished, the inhibitive effects of the protocol last for up to one hour. Although it is not entirely clear how and why TBS works as efficiently as it does, it has been used in a growing number of TMS studies since its discovery, as well as in clinical settings, for the obvious reason of its relatively long lasting after effects.

Limitations of TMS

Like any of the previously described methods, TMS is characterised by great benefits but also by several limitations, which should be taken into account when designing a study, as well as when interpreting results of TMS application. The first

concern is its spatial resolution. As a magnetic field is emitted from the surface of the magnetic coil, a large area of the cortex receives magnetic stimulation. This can be advantagous if the aim is to stimulate a large area, as is often the case in clinical applications of TMS. However, if the aim is to apply TMS over a specific brain area and assess the effects of this stimulation on behaviour, stimulating a large part of the cortical surface obscures conclusions about the exact functional relationship between this specific brain area and its effects on behaviour. This is why in cognitive neuroscience studies using TMS most commonly a so-called figure-eight or butterfly coil is employed. Both names refer to the physical shape of this coil type, which consists of two circular coils which slightly overlap. On account of addition of the magnetic fields produced from the two circles, magnetic field strength in a limited spatial area directly underneath the overlapping parts of these circles is hugely increased, created a more focal stimulation surface, hence improving spatial resolution. It should be noted that brain areas underlying the non-overlapping sections of the circles might still receive some magnetic stimulation, albeit to a much lesser extent.

Hence, the effective spatial resolution of TMS in terms of cortical surface stimulated can be improved by exploiting the additive properties of magnetic fields to create a more focal magnetic peak. However, the magnetic field produced by the coil is not only applied in horizontal directions, it also spreads in the vertical direction. After passing through the skull, the magnetic field does not suddenly stop once it reaches the cortex. Instead it further penetrates the brain, and the further the distance from the origin of the field, the weaker and the less focal it gets. This implies that besides the specific cortical area which was initially aimed for, neighbouring or more deep-down brain areas and structures might receive some degree of magnetic stimulation as well. To reduce the influence of this possible confound as much as possible, it is important to apply TMS only while holding the surface of the coil directly on the scalp, and moreover, perpendicular to it in all directions. Another implication of the fact that magnetic fields spread out and logarithmically lose strength as distance from the source increases, is that only superficial - hence cortical - sites can be efficiently targeted with TMS. To target brain structures situated deeper in the brain higher stimulation intensities would be needed, which could compromise participant safety and comfort. But, more importantly, each brain structure lying between the scalp and the designated deeper brain structure would receive stimulation as well – and to a larger extent than the actual target site. Consequently, using currently commercially available setups, only superficial areas of the brain can be targeted with TMS.

Additionally, with the recently emerging technical advance allowing for simultaneous TMS and fMRI applications, it has been shown that magnetic

stimulation does not always stay confined to the area of application. Instead, (de)activating effects of local TMS might spread to remote cortical areas (Bestmann, Swayne, Blankenburg et al., 2008; Ruff, Bestmann, Blankenburg et al., 2008; Sack, Kohler, Bestmann et al., 2007). Hence, inhibition of a certain brain area might lead to a change in behaviour, but this might at least partly be related to non-local effects of TMS, elsewhere in the functional network. Consequently, remote effects of TMS might complicate the interpretation of behavioural effects of TMS. However, the same remote effects also offer the unique possibility to identify how functional activation spreads throughout a complex cognitive network.

Another issue related to the use of TMS is the fact that although we aim to stimulate certain brain areas, from the outside we are only able to see the scalp, not the underlying anatomical or functional brain layout. This fact might further limit the effective spatial resolution of TMS, since we might be stimulating a different brain structure than the one originally intended. There are several ways to circumvent this issue. One option is to mark stimulation sites according to their location on the skull, most commonly according to the 10-20 EEG positioning system. Several easily detectable landmarks on the skull such as the naseon and the inion are marked, relative to which certain standard positions are determined. This method provides some inter-subject objectivity for coil positioning, and a coarse estimate of which brain structures might underlie each position, but since anatomical and functional layout differ across individuals it is not a very precise method. Another option is to use neuronavigation, a system which links an MRI-based reconstruction of the skull and brain of a certain participant to their physical presence, and allows for very precise coil positioning and position maintenance. The target site for TMS can be defined in two ways: based on anatomical landmarks, such as a certain gyrus, or based on functional imaging data. The latter offers the advantage that, in each individual participant, specifically their functional task-related hotspot can be targeted, thus maximising the chances that TMS effectively disturbs task-related processing. Indeed, the advantages of neuronavigation, and especially functional neuronavigation, over landmark-based positioning have been quantified in terms of increased statistical power, and thus requirement of a smaller sample of participants (Sack, Cohen Kadosh, Schuhmann et al., 2009). On the other hand, MRI scanning is a time-consuming and costly business, and functional imaging might not be feasible or necessary in each situation and with each experimental design.

A further issue concerning TMS, is that the exact way in which the magnetic field influences cortical processing is yet unclear. Animal studies investigating the neural effects of TMS did reveal its basic mechanisms of action, but are not always informative when it comes to the more complex human brain.

Di Lazzaro and colleagues (2003) were previously able to show how various types of TMS pulses differentially affect initial and subsequent volleys of activation descending along cortical axons towards the spinal cord. However, such opportunities for studying physiological effects of magnetic stimulation on the functioning human brain are very limited, and the complexity of the brain hinders simple interpolation of previous findings to different brain areas. In addition, information about physiological effects of TMS not always translates into easily applicable practical recommendations for the use of TMS. For example, it has been shown that using different waveforms of TMS (monophasic versus biphasic) might affect the manner or layout in which cortical neurons are affected (Arai, 2005), but it is not yet clear how to practically deal with such facts, other than keeping stimulation parameters stable across participants.

Finally, an important factor to consider when applying TMS is participant safety.

TMS safety

Whereas previously fMRI and EEG were described as non-invasive and very safe, TMS falls into a slightly different category. Although it is considered safe to use in healthy participants, there are certain risk factors which should be carefully considered before every instance of TMS application.

Although it is an extremely rare event, the application of TMS pulses could induce an epileptic spell in the participant. Over the course of twenty years and tens of thousands of people undergoing TMS, seizures have been induced in a very limited number of occasions, most of which could post hoc be retraced to the presence of certain risk factors. Those participants who experienced a seizure did not experience any health related effects outlasting the spell itself. The sheer rarity of seizures occurring makes it impossible to quantify how high a risk actually is, and if and how risk factors add up or interact. But in case of experimental research, in which application of TMS does not hold any benefits for the participant, it is common practice not to take any unnecessary risk. Firstly, using a repetitive protocol increases the risk of inducing seizure, and the more pulses are applied, the higher the risk. In addition, the higher the intensity with which these pulses are applied, the higher the risk. Besides these stimulation parameters, several risk factors can be identified related to the background and current state of the participant. For example, a (family) history of epilepsy is considered a risk factor, as are sleep deprivation, the use of certain medications such as antihistamines and antibiotics, alcohol, and drugs.

In order to ensure participant safety, it is the responsibility of each TMS laboratory to create a procedure which limits the risk of inducing seizure as much

as possible. At the Maastricht Brain Imaging Centre (M-BIC), every potential participant is screened via an extensive questionnaire, which is subsequently checked and, if none of the exclusion criteria are met, approved by a medical doctor. Prior to each application of TMS the participant completes an additional, shorter questionnaire, which provides the experimenter with relevant information about the current state of the participant, such as if this person is well-rested and currently not on any medication. Only if these criteria are met as well, TMS will be applied. Application of TMS is restricted to certified users, which have undergone theoretical and practical training concerning safe use of TMS. Each TMS study has to be submitted for approval to the medical ethical committee of the Maastricht University Medical Centre.

Combining different methods of cognitive neuroscience

In the previous section the methods of fMRI, EEG/ERP and TMS have been described. Each described method has its strong points and its weaknesses. However, combining several of these methods offers the potential to fully exploit the benefits of each individual method, while compensating for some of their shortcomings. The work described in this thesis incorporates different methodological approaches, and in most cases these approaches were combined in an effort to combine the best of two worlds.

FMRI and TMS can be combined in several ways. Firstly, information about the cortical layout of certain cognitive functions, obtained with fMRI, can be used as input for a TMS follow-up study, which offers the potential of verifying the actual functional relevance of the identified areas (Chapter 2). The same procedure can be applied based on outcomes obtained from connectivity analyses (Chapter 2), possibly to verify the flow of information through the network using time-resolved TMS. Frameless stereotactic neuronavigation allows for positioning of the TMS coil exactly at individual functional hotspots, and verifying during data collection that the stimulation sites remain the same throughout the experiment. This approach hugely increases the precision and thus the statistical power of TMS experiments (Sack et al., 2009). Yet, it does not permit observations of how TMS affects ongoing cortical processing.

A second way to combine fMRI and TMS, as an extension of the previously described neuronavigation based on individual functional imaging data, is to use a long lasting stimulation protocol like cTBS and subsequently visualise its effects on fMRI data, as well as on behavioural performance (Chapter 5). Importantly, this approach not only informs us about the brain changes corresponding to changes in

behavioural performance. It also provides information about instances in which TMS does not lead to behavioural changes, but might nevertheless influence cortical processing, for example when functional disruption of a brain area is instantaneously compensated by a different brain area in the network. This combination of TMS and fMRI has the potential to reveal unprecedented and important information about what is actually going on in the brain during application of TMS. A similar approach can of course be employed using ERP instead of fMRI (Chapter 4). By using long lasting cTBS stimulation and subsequently recording ERPs during task execution, temporal aspects of altered task processing and performance due to TMS disruptions can be visualised.

In addition to these offline combinations of methods, it is now also possible to simultaneously combine two of the aforementioned methods, or even all three of them. These simultaneous combinations offer the prospect of great advances, both in understanding the fundamental mechanisms of TMS, and in unravelling the neural correlates underlying cognitive functioning. However, the highly magnetic environments created by fMRI and TMS are potentially detrimental to EEG acquisition, which is sensitive to electrical and magnetic noise. Also, TMS in the MR scanning environment is associated with certain methodological challenges. However, it has already been shown that these issues can be dealt with, for example by appropriate filtering and interleaved recordings schemes. Currently, our group is succesfully conducting simultaneous TMS-fMRI, TMS-EEG, and even TMS-EEG-fMRI measurements at the M-BIC facilities.

Parietal cortex and spatial cognition

In most of the following chapters, several of the discussed methods were combined in order to investigate the recurrent theme of spatial cognition, thereby focussing on its main underlying neural correlate, the posterior parietal cortex (PPC). The term spatial cognition broadly refers to a collection of abilities and processes dealing with spatial information (Sack, 2009), which is both arising from external sources via sensory input, as well as from internal sources such as proprioception and motor efference copies. It includes not only physical spatial information such as relative spatial locations and distances, but also more abstract representations which are to some extent spatially organised, such as sequence, size, numerical value and quantity (Chapter 4 and 5). In addition to processing incoming spatial information, parietal cortex also has the ability to exert influence on remote brain areas by sending top-down feedback signals, for example guiding attention or motor actions (Chapter 3). And finally, there are also frequent

interactions between these processing and influencing abilities of parietal cortex during spatial cognition, whereby incoming spatial stimuli are for example able to influence spatial attention and thus perception of other stimuli (Chapter 3 and 4).
In line with the diversity and pervasiveness of the functions subserved by PPC, its activation is reported in many different functional imaging studies. Yet, although PPC seems to be implicated in almost any complex cognitive task, the actual functional contribution and relevance of these activations are much less frequently addressed. A possible explanation for this underexposure of the functional layout of PPC is that highly complex cognitive skills and processes are not easy to investigate, and might often require a more elaborate methodological approach when simply contrasting several conditions with each other is not sufficient. In addition, it is known that on top of individual differences regarding brain anatomy, the functional organisation of the PPC can strongly differ between individual participants, which might be linked with the fact that complex functions such as those carried out by PPC are in many cases shaped by experience and sensory input, and might thus develop slightly different in each person. As a result, group averaging which is commonly employed to gain statistical power might result in spatially different functional activation spots cancelling each other out. Furthermore, in many cases, PPC might serve as a modulator or mediator of functions represented in remote brain areas and networks, rather than represent a separate functional module. The complex cognitive functions supposedly subserved by PPC actually involve distributed processing within functional networks, whose dynamics influence PPC, and vice versa.

Another striking characteristic of PPC, and one recurring in several of the following chapters, is the hemispheric asymmetry regarding the neural correlates of spatial cognition (Sack, 2009). There seems to be a division of labour between the left and right hemispheric counterparts of PPC, and although the exact layout of the division is not yet clear, there is ample evidence for a right-hemispheric dominance in spatial cognition. Healthy adults display an attentional bias towards the left, the so-called pseudoneglect, implying a stronger influence of the right hemisphere (Orr & Nicholls, 2005). Deficits of spatial attention, such as visual hemineglect, are much more frequently reported as a result of right parietal lesions (thus affecting the right visual field) than of left (Vallar & Perani, 1986). Since there is no reason to assume that right parietal cortex is more often damaged than left, this imbalance must rather reflect an asymmetrical expression of parietal lesions, with right parietal lesions leading to cognitive impairment more often. In healthy adults similar lateralisations have been demonstrated, for example when mimicking the syndrome of hemispatial neglect using TMS (Hilgetag, Theoret, & Pascual-Leone, 2001) or in the context of spatial mental imagery (Sack,

Camprodon, Pascual-Leone, & Goebel, 2005; Sack, Sperling, Prvulovic et al., 2002).

Several competing theoretical accounts have tried to explain this hemispheric asymmetry. According to the theory of *hemispheric asymmetry in attention*, the left hemisphere controls attention only in the right hemifield, whereas the right hemisphere is able to control both sides (Corbetta, Miezin, Shulman, & Petersen, 1993). This would imply that the right hemisphere is able to compensate for a lesion in the left hemisphere, but not vice versa, which is in line with the results of several empirical studies (Sack et al., 2005; Sack et al., 2007; Sack et al., 2002) (Chapter 5). Alternatively, the *opponent-processor model* proposed by Kinsbourne (1993) claims that the attention of each hemisphere is biased in the direction of the contralateral hemifield, with the rightward bias of the left hemisphere being stronger. Both hemispheres mutually inhibit each other's orienting biases, leading to stable, slightly leftward biased allocation of spatial attention across space. This balance remains until one of the two hemispheres is damaged, when the healthy hemisphere exerts more inhibition on the damaged one, thus shifting the attentional gradient. In line with Kinsbourne's model, TMS induced disruption over both parietal cortices in healthy volunteers restored the attentional balance which was previously disturbed by unilateral TMS (Dambeck, Sparing, Meister et al., 2006) (Chapter 3). Hence, while claiming different underlying physiological mechanisms, both theories account in their own way for the observed hemispheric asymmetry with regard to spatial cognition. This makes it difficult to distinguish between the different models at a behavioural level. Additional methods and measures such as TMS and brain imaging might further clarify this issue.

Summarising, the parietal cortex, and specifically the PPC, constitutes a fascinating, yet underexplored area of the brain. In the work described in the following chapters we aimed to explore how several different elements of spatial cognition are represented by the PPC and functionally connected networks. To this end we employed combinations of well-considered behavioural designs with several methods of cognitive neuroscience, thereby exploiting the benefits of one method to compensate for the limitations of another.

Research aims and thesis outline

The general aim of the work described in the subsequent chapters was to explore how functional brain networks involved in higher cognition can be revealed, probed and perturbed using different combinations of research methods and analysis approaches. More specifically, in most of the following chapters, there is a focus on how different aspects of spatial cognition are subserved by networks revolving around the posterior parietal cortex.

Whenever we observe a movement of a conspecific, our mirror neuron system becomes activated, urging us to imitate the observed movement. Supposedly, an inhibitive component keeps us from imitating everything we see. The aim of *Chapter 2* was to unravel the neurodynamics underlying this proposed inhibition of automatic imitation. This aim was pursued by visualising the involved networks using fMRI and effective connectivity analysis, and subsequently manipulating the integrity of the revealed network during task execution, using TMS. As a result, the presence of a final gating mechanism allowing for inhibition of automatic imitation could for the first time be substantiated with empirical evidence.

The aim of *Chapter 3* was to further investigate the aforementioned hemispheric asymmetry regarding spatial cognition, and probe whether right parietal cortex is indeed more vulnerable to spatial deficits, as suggested by lesion studies. The neuropsychological syndrome of contralesional extinction was mimicked, by temporarily inducing a spatial attentional bias in healthy volunteers with TMS. Subsequently, it was investigated whether directing covert spatial attention could enhance or, more importantly, counteract the resulting behavioural deficits.

In *Chapter 4* the influence of multisensory interactions and synesthetic associations on spatial localisation is explored. The hypothesis is put forward that widespread, subtle forms of synesthesia provide crossmodal mapping patterns which underly and influence multisensory perception. By combining psychophysics, TMS and ERP recordings, we investigated whether the neural basis of pitch-size synesthesia crucially depends on normal multisensory processes, mediated by right posterior parietal cortex.

Chapter 5 zooms in on a different aspect of spatial cognition, namely spatial mental imagery, in an attempt to visualise cortical compensational mechanisms. Spontaneous brain plasticity occurring after a brain is lesioned can contribute greatly to patient rehabilitation. Yet, the mechanisms underlying this process are still elusive. Long-lasting patterned TMS over left or right parietal cortex was combined with subsequent fMRI, during which a spatial mental imagery

task was executed. The results presented in this chapter substantiate how the brain can instantaneously re-route functional activations in order to maintain behavioural performance.

In the final *Summary and Conclusions* chapter, the insights gathered from the previous chapters are summarised and combined, and directions for future research are presented.

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Chapter 2

The brain's intention to imitate

The neurobiology of intentional versus automatic imitation

Based on:
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Abstract

Whenever we observe a movement of a conspecific, our mirror neuron system becomes activated, urging us to imitate the observed movement. However, since such automatic imitation is not always appropriate, an inhibitive component keeping us from imitating everything we see seems crucial for an effective social behaviour. This becomes evident from neuropsychological conditions like echopraxia, in which this suppression is absent. Here, we unraveled the neurodynamics underlying this proposed inhibition of automatic imitation by measuring and manipulating brain activity during the execution of a stimulus-response-compatibility paradigm. Within the identified connectivity network, right middle/inferior frontal cortex sends neural input concerning general response inhibition to right premotor cortex, which is involved in automatic imitation. Subsequently, the fully prepared imitative response is sent to left opercular cortex which functions as a final gating mechanism for intentional imitation. We propose an informed neurocognitive model of inhibition of automatic imitation, suggesting a functional dissociation between automatic and intentional imitation.

Introduction

Whenever we observe a movement of a conspecific, our mirror neuron system becomes activated, urging us to imitate the observed movement. Action observation leads to an activation in the (pre)motor cortex normally involved in the execution of the same action, a process facilitated by mirror neurons; motor neurons that also respond to sensory information concerning biological motion (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Iacoboni, Koski, Brass et al., 2001; Iacoboni, Woods, Brass et al., 1999; Rizzolatti & Arbib, 1998). This motor activation induces a tendency to imitate the observed action (Buccino, Vogt, Ritzl et al., 2004; Chaminade, Meltzoff, & Decety, 2002; Chaminade, Meltzoff, & Decety, 2005; Decety, Chaminade, Grezes, & Meltzoff, 2002), a neural mechanism considered to be involved in an array of processes ranging from simple action recognition and action understanding, to high cognitive functions such as language, theory of mind, social behaviour, or even disorders like autism (Blakemore & Decety, 2001; Keysers, Wicker, Gazzola et al., 2004; Rizzolatti et al., 1998; Umilta, Kohler, Gallese et al., 2001; Wicker, Keysers, Plailly et al., 2003).

Numerous previous studies have aimed at revealing the exact neural mechanisms underlying our natural tendency for imitation (Buccino, Binkofski, Fink et al., 2001; Fadiga et al., 1995; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Grafton, Fadiga, Arbib, & Rizzolatti, 1997; Heiser, Iacoboni, Maeda et al., 2003; Iacoboni et al., 2001; Iacoboni et al., 1999; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). However, the potential existence of an equally important and closely related neural mechanism enabling us to inhibit such automatic imitative response tendencies has been largely neglected. In many daily life situations, however, such suppression is crucial for an effective social behaviour, e.g. when action imitation is not appropriate or even maladaptive.

Neuropsychological support for the existence of such an inhibitory mechanism of automatic imitation comes from a condition in which imitation is positively disturbed. Luria described a condition called echopraxia, in which patients suffering from frontal lobe damage repeated every observed movement, preferably in a mirror-image fashion (Luria, 1966). Lhermitte reported that many frontal syndrome patients showed 'obstinate imitation behaviour'; a severely increased urge to imitate every observed action, even if they were explicitly instructed not to do so (Lhermitte, Pillon, & Serdaru, 1986). Even though these patterns of behaviour seem similar in nature, since they both involve an increased urge to imitate observed movements, Lhermitte explicitly classifies them as different conditions. Whereas echopraxic imitation is immediate, involuntary, with the speed and abruptness of a reflex, and not relying on intellectual processes,

obstinate imitation behaviour is a voluntary act of higher cognitive nature. Lhermitte (Lhermitte et al., 1986) proposes that damage to the anterior, inferior part of the frontal lobe results in a release of inhibition of activity from the parietal lobe, causing inappropriate imitation behaviour to occur. Notwithstanding the possible differences, both conditions involve severely increased imitative behaviour, and both are being linked to frontal lobe damage. The implications following from these phenomena of increased imitation are twofold: firstly, in accordance with mirror neuron theory, there is an automatic tendency to imitate observed actions, and secondly, under normal physiological circumstances, this tendency can be suppressed if needed.

Traditionally, processes of response inhibition have been investigated using overlearned responses, in paradigms such as the well-known Go-Nogo or Stroop tasks. In accordance with lesion studies, brain imaging studies reveal a network of several frontal and cingulate cortical regions involved in response inhibition. Using the Stroop task, areas in the left inferior frontal gyrus (Langenecker, Nielson, & Rao, 2004), right lateral prefrontal cortex (Vendrell, Junque, Pujol et al., 1995), prefrontal and anterior cingulate cortex (Harrison, Shaw, Yucel et al., 2005), superior and inferior parietal lobules, and inferior frontal gyrus and extrastriate cortex (Milham, Erickson, Banich et al., 2002) were found to be of importance for the inhibition of the overlearned reading response. Using the Go-Nogo task, Dillon and Pizzagalli (Dillon & Pizzagalli, 2007) concluded that response inhibition depends upon fronto-basal ganglia networks, and that right ventrolateral prefrontal cortex may support a general inhibitory process. Horn and colleagues (Horn, Dolan, Elliott et al., 2003) found support for the hypothesis that the anterior lateral orbitofrontal cortex is activated during response inhibition. They also concluded that a network of higher order association and paralimbic areas is involved in response inhibition. In an fMRI and connectivity study, Koechlin and colleagues (Koechlin, Ody, & Kouneiher, 2003) report that caudal lateral prefrontal regions are involved in selecting premotor representations associating stimulus and motor responses.

Most studies investigating response inhibition focus on a single paradigm. However, the downside of this approach is that it is difficult to disentangle activation caused specifically by response inhibition from complex, task specific activations. In an attempt to specify brain regions involved in response inhibition in general, Wager and colleagues (Wager, Sylvester, Lacey et al., 2005) compared fMRI activation and connectivity patterns resulting from three different inhibition tasks: a go/no-go task, a flanker task, and a stimulus-response compatibility task. They found a set of frontal and parietal regions commonly engaged in response inhibition across the three tasks, including bilateral anterior insula / frontal

operculum and anterior prefrontal, right dorsolateral and premotor, and parietal cortices. Even though correlations among tasks both for brain activity and performance were low, probably because of individual biases unique to each task, they suggested that certain common interference detection and/or resolution mechanisms are engaged across tasks.

Only few neuroimaging studies have attempted to specifically identify the neural correlates underlying the inhibition of imitative response tendencies in healthy human volunteers. Brass and colleagues (Brass, Zysset, & von Cramon, 2001b) explored the cortical mechanisms underlying the inhibition of imitation in an event-related functional magnetic resonance imaging study (fMRI), using a simplified stimulus-response paradigm, a task frequently used in studies mapping the human mirror neuron system (Brass, Bekkering, Wohlschlager, & Prinz, 2000). A lack of activation in regions usually detected in inhibition studies, including the inferior frontal gyrus and the anterior cingulate cortex, was interpreted by the authors as indirect evidence that the inhibition of imitative responses employed different cortical structures than inhibitive mechanisms involved in Go-Nogo and Stroop tasks. A second fMRI study aimed at finding a functional dissociation between the inhibition of overlearned and imitative responses (Brass, Derrfuss, & von Cramon, 2005). The authors indeed showed this dissociation, supporting the hypothesis that the inhibitive mechanisms in both processes consist of different components. In addition, this study revealed a region in the right inferior frontal gyrus that seemed to be involved in both inhibitive processes. The authors reasoned that this region is involved in generating a stop signal right before the response is executed. Thus, even though the cortical mechanisms preceding this decision are different, the eventual inhibition of the movement could be executed by a mutual region.

The results of these studies are insufficient to explain the process of the inhibition of imitation. Partly this can be ascribed to the experimental design of the aforementioned studies. Brass and colleagues (Brass et al., 2001b) compared cortical activity in response to execution of a finger action while participants were observing either the same (congruent) or a different (incongruent) action on a screen. The congruent condition was supposed to reflect imitation, the incongruent condition should reveal inhibition of an imitative response. However, in this design actions were not actively imitated, or inhibited, for that matter. Rather, stimulus and response merely either coincided, or not, which is not the same as intentionally imitating an observed action. In the second study (Brass et al., 2005), besides the previously described task a Stroop task was used to detect functional dissociations between the inhibition of imitative and overlearned responses. However, since these tasks were utterly different, the resulting cortical activations

could not be compared directly. Rather, congruent and incongruent conditions were contrasted for both tasks, and the produced cortical areas were merely compared qualitatively.

Besides design shortcomings, there are methodological issues. Using exclusively fMRI to investigate processes of response inhibition certainly has its limitations. The BOLD signal allowing for visualisation of local brain activity is likely to represent intracortical processing rather than neuronal spiking rate (Logothetis, Pauls, Augath et al., 2001b; Logothetis & Wandell, 2004). An area influencing inhibition might thus appear in the functional image in an identical manner as an excited area, namely as an area of increased synaptic activity. Indeed, it seems that complex inhibition can be reflected in a larger, smaller, or no BOLD signal at all (Ritter & Villringer, 2002). Evidence supporting the notion of negative BOLD deflections reflecting decreased neuronal activity (Shmuel, Augath, Oeltermann, & Logothetis, 2006) and/or cortical inhibition (Hummel, Andres, Altenmüller et al., 2002; Hummel, Saur, Lasogg et al., 2004; Kastrup, Baudewig, Schnaudigel et al., 2008) has also been presented recently. However, the exact significance of negative BOLD responses is not yet known, and as of yet there is no proof that this mechanism plays a role in the inhibition of automatic imitation.

Although investigating response inhibition using functional imaging appears to be suspect to ambiguous signal interpretations, sensible hypothesizing and, consequently, construction of a clear cognitive model prior to experimental recording can lead to simple, testable predictions. In the current study, as in most studies concerning response inhibition, we considered inhibition an active process, resulting in an increase in BOLD signal. In addition, methods investigating connectivity and causality in the brain can be of great use when investigating processes of inhibition. Data-driven effective connectivity analysis is capable of revealing targets and sources within fMRI data, without having to rely on a priori specification of a model that contains pre-selected regions and connections between them. It provides temporal information about the direction of information flow through an activated network.

Using effective connectivity analysis, conclusions can be drawn with regard to the probable directed and task-related neural influence one cortical region exerts over another one (Friston, Holmes, Poline et al., 1995), making it particularly suited for questions regarding cortical inhibition. However, it does not provide evidence with regard to causal relationships between brain activation and behavioural output. Brain areas showing up in effective connectivity maps are – at least - correlated to task execution, but whether they are functionally relevant for the actual performance cannot be assessed on the basis of these data alone.

A method particularly useful for investigating causal relationships between activity modulations in specific brain areas and actual behaviour, and therefore potentially also for clarifying issues on cortical inhibition, is transcranial magnetic stimulation (TMS) (see e.g. Hallett, 2000; Pascual-Leone, Walsh, & Rothwell, 2000). By intervening with the functionality of a certain brain region, and at the same time observing possible changes in behavioural responses, assumptions about the causal relevance of a certain brain area for the execution of a certain task can be experimentally tested.

In the current study, the existence, network connectivity, and functional relevance of the specific brain system enabling us to inhibit responses, particularly automatic imitative response tendencies, were investigated. Our explicit goal was to identify, functionally dissociate and manipulate the neural correlates of this proposed inhibition of automatic imitation by using an elaborate combination of complementary non-invasive brain imaging and brain interference techniques.

A stimulus-response compatibility design was employed assessing imitation and inhibition behaviour. During brain imaging, participants had to imitate an observed finger movement while ignoring a simultaneously presented spatial cue (imitative trials), or move their finger as it was indicated by the spatial cue while ignoring finger movements (spatial trials). The imitative and the spatial cue could either appear on the same finger (congruent condition) or on different fingers (incongruent condition). We hypothesized that incongruent trials would mainly reflect response inhibition, and that the condition in which a movement of the "wrong" finger would have to be suppressed (spatial incongruent) would reflect the inhibition of imitation, specifically.

Random effects statistical activation maps were produced to allow mapping of the brain regions involved in response inhibition, and specifically in the inhibition of imitative responses. In order to reveal the direction of information flow within the revealed network, random effects effective connectivity analyses were conducted using the concept of Granger causality (Granger, 1980), producing cortical maps depicting effective connectivity, consisting of source and target activations. Source activations represent regions whose activation consistently predicts future activation (i.e. next timepoint) of the seed region, and thus are hypothesized to have exerted task-related causal influence on the cortical activation of the seed region. Target activations on the other hand represent regions whose activation is consistently predicted by the activation of the seed region (i.e. previous timepoint), and thus are hypothesized to be causally influenced by the seed region. Finally, fMRI-guided TMS was applied to identify the underlying functional dissociation, by revealing the specific functional contribution of each involved brain area for successful imitation and inhibition of imitation.

The converging evidence enabled us to propose a new neurobiological model of the inhibition of imitative response tendencies, distinguishing between automatic and intentional imitation.

Methods

Participants

A total of 15 healthy volunteers with normal or corrected to normal vision (mean age: 23 ± 1.5), 5 of which were male, participated in the fMRI part of this study. 8 of these participants (mean age: 22.3 ± 2.6), 3 of which were male, subsequently underwent TMS stimulation. All participants were unaware of the goal of the study until after having completed their participation. Ethical approval was given by the local medical ethical committee.

Experimental design

Each stimulus consisted of a picture of a hand, followed by an imitative cue (a downward movement of either index or middle finger) and a spatial cue (a small white cross appearing underneath the index or middle finger), emerging at the same time. Subjects rested their index and middle fingers on their respective buttons, waiting for the appearance of the cues to indicate a response with either the index or the middle finger. Depending on the instruction, subjects were required to imitate the observed finger movement while ignoring the spatial cue (imitative trials) or move their finger as it was indicated by a spatial cue while ignoring finger movements (spatial trials). Please note that both responses involved active downward motion of the index or middle finger to press a button, while during baseline subjects' fingers were resting on the response buttons without active extension of the fingers. The imitative and the spatial cue could either appear on the same finger (congruent condition) or on different fingers (incongruent condition) (see Figure 1 or the stimulus material section for more details). A 2 by 2 design was constructed, containing the independent variables "congruency" and "target".

fMRI design

A fast event-related design was chosen for the fMRI study. A blocked presentation was necessary with regard to the "target" condition, because the imitative and spatial trials required different task instructions for the subject. A psychophysical pilot study, however, revealed that a blocked design would be too predictable to

disclose congruency effects. Consequently, a mixed blocked and event-related fMRI design was applied.

A total of 256 trials, 64 per condition, were assigned to 2 functional acquisition runs containing 8 blocks each. Each block contained 16 trials. The 8 blocks, presented in semi-randomized order, consisted of 4 imitative and 4 spatial blocks, all containing both congruent and incongruent trials in randomized order.

Each block started with a 22.5 second period of fixation, visual instruction, and again fixation. Stimulus onset asynchrony, which was varied in a semi-random manner, varied between 4500 and 9000 ms (3, 4, 5 or TR's). This added up to a block duration of almost 2 minutes and 50 seconds. The duration of a run was 22 minutes and 40 seconds, resulting in an experimental duration of 45 minutes.

TMS design

Event-related TMS was applied with a figure-of-eight TMS coil, using 10 Hz triplets, starting at 200 ms after the onset of the imitative and spatial cue. Subjects were

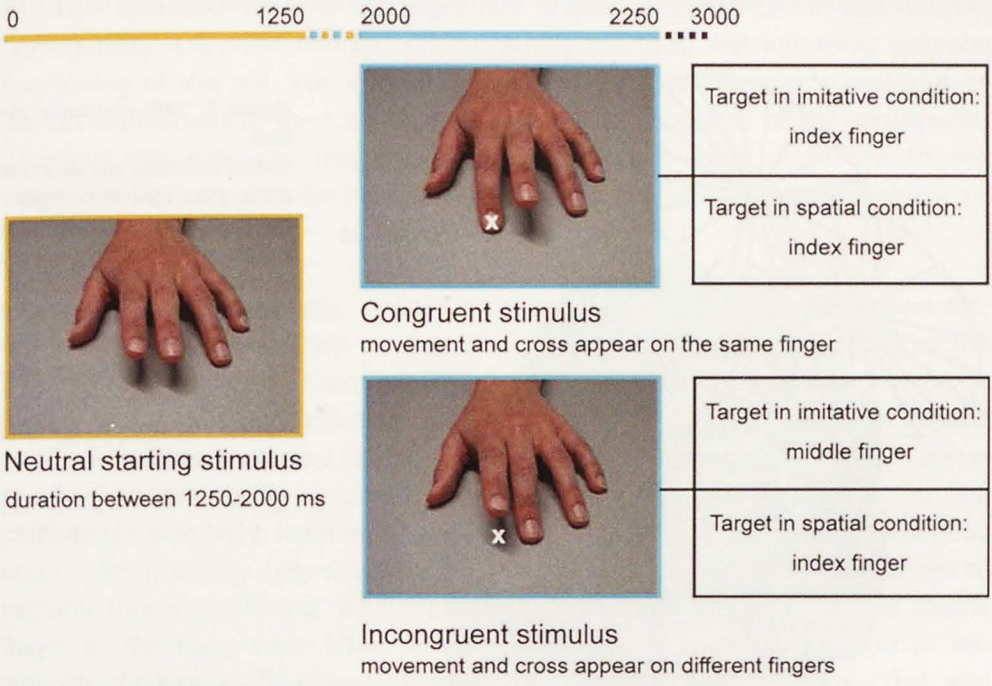

Figure 1. Stimulus-response compatibility design
The stimulus-response compatibility design relies on the assumption, as put forward by mirror neuron theory, that the observation of a movement evokes a simulation of this movement in the observers motor cortex. This projection results in an urge to imitate the observed movement. The paradigm contains conditions in which this imitation is permitted, and conditions in which it has to be suppressed.

stimulated at 120% of their resting motor threshold (rMT). The experiment consisted of 3 sessions, during each of which a single target site was stimulated. Each session contained 8 blocks, during 4 of which participants received magnetic stimulation. The resulting 4 non-stimulation baseline blocks were alternated with stimulation blocks. An imitative TMS block was followed by an imitative non-TMS block, or vice versa. Each block contained 12 trials with a duration between 6000 and 7000 ms.

Three target sites were selected based on fMRI group data. Two of these regions derived from functional brain imaging data. The third was detected as a result of effective connectivity analysis. For each participant, these target sites were detected within their individual brain imaging data set, using five selection criteria. Firstly, the candidate region had to be directly accessable with TMS (Sack & Linden, 2003). Secondly, using the same contrast that yielded this particular target region in the group data, the candidate target region had to show considerable activation in each single subject analysis. Thirdly, it had to display a BOLD-response signal consistent with the conducted contrast analysis (deconvolution timecourses), fourthly the BOLD signal characteristics had to be

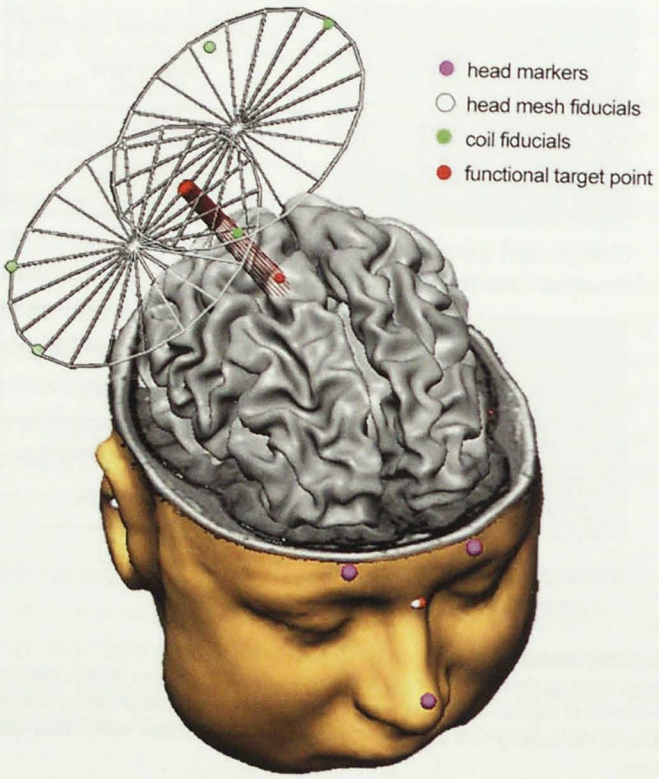

Figure 2. TMS neuronavigation
Several landmarks and ultrasonic markers are used to co-register the TMS coil to the (f)MRI data and the actual physical presence of the participant. Once co-registered, the system continuously provides information about the position of the TMS coil relative to the selected target site, online and at millimeter precision level.

comparable to those resulting from group data analysis, and finally, stimulating the target region should not directly interfere with right hand motor execution.

Participants were co-registered to their structural brain imaging data using the BrainVoyager TMS Neuronavigator (Brain Innovation BV, Maastricht, The Netherlands), a system that allows for continuous online coil navigation directly above a cortical target point localized using fMRI activation (Figure 2). TMS target points were defined on the cortical surface using individual functional brain imaging data (Figure 3). The figure-of-eight TMS coil was mounted in an adjustable arm, and positioned directly over the intended target stimulation site, tangentially to the participant's skull in both the horizontal and vertical dimension. During the course of the stimulation sessions, the proximity of the coils "hotspot" to the target point was continuously monitored and, if necessary, the position was adjusted online. Subjects were positioned in a head and chin rest. Coil orientation was kept as constant as possible between subjects. For the right premotor and middle frontal gyrus target points, the coil was oriented in a 45 degree angle respective to the longitudinal fissure, with the coil handle pointing in a posterior-superior direction. For the left opercular region preservation of inter-subject homogeneity was more difficult, since not only the head rest interfered with the positioning of the coil, but also some subjects experienced muscle reactions to certain orientations of the coil above this region. In almost all of the participants, however, the coil was positioned in a 90 degree angle respective to the longitudinal fissure, with the handle pointing in the medial-superior direction.

Stimulus material

During the complete study, Presentation software (Neurobehavioural Systems, Inc., Albany, USA) was used for both stimulus presentation and recording of the behavioural responses. Each experimental stimulus started with a stationary picture of a hand, recorded from a viewing point such that it looked as the left hand of another individual sitting across from the subject (Figure 1). Like in previous studies using a similar design, a stimulus mirror imaged to the participants own right hand was used, since this is the setting in which imitation occurs mostly during daily social engagements, and in which imitation is easiest to perform (Brass, Bekkering, & Prinz, 2001a; Luria, 1966). The index and middle finger of the hand were lifted, in order to correspond to the position of the subjects' fingers as they were held above their respective buttons. This was important to ensure that the observed movement would indeed correspond to the executed movement, thus ensuring stimulus-response compatibility.

After a randomly varied period of either 1250, 1500, 1750, 2000 or 2250 ms, the imitative and spatial cues appeared (Figure 1). A picture of the same hand

replaced the initial picture, except now showing either the index or the middle finger in a downward position, while the rest of the hand and background remained the same. At exactly the same time, the spatial cue, consisting of a small white cross, appeared. In congruent trials this cross appeared on the same finger that had made the downward movement. In incongruent conditions the cross appeared on the finger that remained stationary.

As stated earlier, a picture of the final posture of the "moving" finger followed the picture of the static hand, thus creating a powerful illusion of motion. Movie recordings of the same movement could have resulted in slight differences in the starting position, as well as the onsets of the trajectory of the different finger movements. Also, it would have created an imbalance between the imitative and the spatial cue: whereas the appearance of the movement would have started gradually and become more noticeable every few milliseconds, the cross would have appeared from one moment to the other. Timing those events such that they would be equivalent would have been impossible. Hence, the use of static pictures replacing each other was preferred over the use of a movie, to ensure maximum controllability with regard to cue onset and duration. Participants consistently reported that they did not notice seeing two consecutive pictures, instead of a movie of a natural movement.

Both the imitative and the spatial cue remained visible for 1000 ms. Trial duration was fixed at 3500 ms. In between trials, subjects were shown a white fixation cross on a monochrome black background.

Apparatus and data acquisition

fMRI acquisition

Brain imaging data were gathered using a 3 Tesla Siemens Allegra MR scanner and a volume head coil at the Faculty of Psychology of Maastricht University, The Netherlands.

Functional images were acquired using single-shot gradient-recalled echoplanar imaging (EPI, 710 volumes, TR = 1500 ms, TE = 30 ms, flip angle = 90°). 24 oblique slices were acquired without inter-slice gap. Voxel size was 3.5 x 3.5 x 4 mm^3, and each slice contained a matrix of 64 by 64 voxels.

High-resolution (voxel size 1 x 1 x 1 mm^3) 3D anatomical data were collected using a T1-weighted MDEFT sequence. This sequence consisted of 176 sagittal slices of 1 mm thick and a matrix of 256 by 256 voxels (TR = 7.92 ms, TE = 2.4 ms, flip angle = 15°).

Stimulus material was displayed onto a frosted screen, positioned at the rear end of the scanner bore, using an LCD projector (PLC-XT11-16, Sanyo North

America Corporation, San Diego, USA). An adjustable mirror mounted on the head coil allowed each subject a complete view of the display. Responses were recorded using a standard MR compatible button box (LUMItouch keypads, Photon Control, Burnaby, Canada).

TMS apparatus

Biphasic magnetic stimulation was generated using a Medtronic MagPro X100 stimulator (Medtronic Functional Diagnostics A/S, Skovlunde, Denmark). Magnetic pulses were delivered with a figure-eight-coil (Magnetic Coil Transducer MC-B70, Medtronic), mounted in an adjustable arm.

Data analysis

Brain imaging data analysis

Functional and anatomical brain imaging data were pre-processed and analyzed offline using BrainVoyager QX (Brain Innovation BV, Maastricht, Netherlands).

The first 5 volumes of each run were excluded to allow for T1 saturation, permitting the T2-weighted MR signal to stabilize. The first functional volume served as a high-contrast prototype to which following functional volumes were aligned during pre-processing. Pre-processing of the functional data existed of slice scan time correction using sinc interpolation, 3D motion correction involving trilinear interpolation, linear trend removal, and application of a 3 cycles per timecourse ($2.83*10^{-3}$ Hz) high pass filter. Individual functional runs were inspected visually to ascertain that subjects had not moved excessively during data recording, which was not the case. Anatomical data were standardized using Talairach transformation (Thalairach & Tournoux, 1988). Subsequently, the 2D functional runs were transformed into Talairized 3D volume timecourses. Volume time courses were spatially smoothed using a 6 mm FWHM kernel.

The statistical analysis of the variance of the blood oxygen level-dependent (BOLD) signal was based on the application of multiple regression analysis to time series of task-related functional activation (Friston et al., 1995). Predictor time course were adjusted for the hemodynamic response delay by convolution with a hemodynamic response function (Boynton, Engel, Glover, & Heeger, 1996) (delta 3, tau 2). Contrasts between conditions were carried out using BrainVoyager's Random Effects (RFX) GLM analysis tool, enabling generalisation of the statistical inferences to the population level. Fixation periods at the start and end of each block were used as a baseline.

To obtain mirror neuron related activation maps, the two experimental imitative conditions were contrasted with the two experimental spatial conditions, since mirror neuron hypothesis would predict that the mirror neuron network is especially activated during instances of imitation.

Processes of response inhibition mutual to both imitative and non-imitative responses were considered congruency main effects, and were visualized by subtracting congruent trials (imitative and spatial) from incongruent trials (imitative and spatial). Particularly important was a contrast which would identify regions specifically involved in the inhibition of imitative responses. The rationale was that observing a finger movement while the task demanded that an adjacent finger was moved in a similar way, as was the case in the spatial incongruent condition, would lead to an urge to imitate, which would have to be actively inhibited to complete the task. Subtraction of the imitative incongruent BOLD-responses from the spatial incongruent ones would reveal areas involved in suppressing the urge to imitate the moving finger. To exclude areas related to response inhibition in general, in addition to a higher response to incongruent spatial trials, the response to imitative incongruent trials should be as low as, or lower than, the response to imitative congruent trials, resulting in the following contrast: (spatial incongruent - spatial congruent) - (imitative incongruent + imitative congruent). This contrast would reveal activation in response to suppressing the urge to imitate, corrected for general incongruency effects.

For visualisation purposes, statistical activation maps were projected onto inflated and flattened template cortices and smoothed once with a repeat value of 5. Cluster size threshold was set at 50 voxels. Results from the Random Effects analysis were reported using p-values. In addition to statistical contrasts event-related deconvolutions were used to visualize relative activations between conditions within a certain cortical region of interest, as produced by statistical analysis.

Functional and effective brain connectivity analysis

Functional connectivity analysis was carried out offline on all 15 subjects, using Random Effects Granger Causality Mapping (GCM, see (Roebroeck, Formisano, & Goebel, 2005) for details), producing cortical maps depicting source and target activations. Random effects analysis enables generalisation of the statistical inferences to the population level.

Three seed regions were selected, two based on their task dependent BOLD characteristics as observed in the group GLM analysis, and one based on one of the produced connectivity maps. Several criteria for the selection of seed regions were employed. Firstly, they appeared as significant activation clusters in

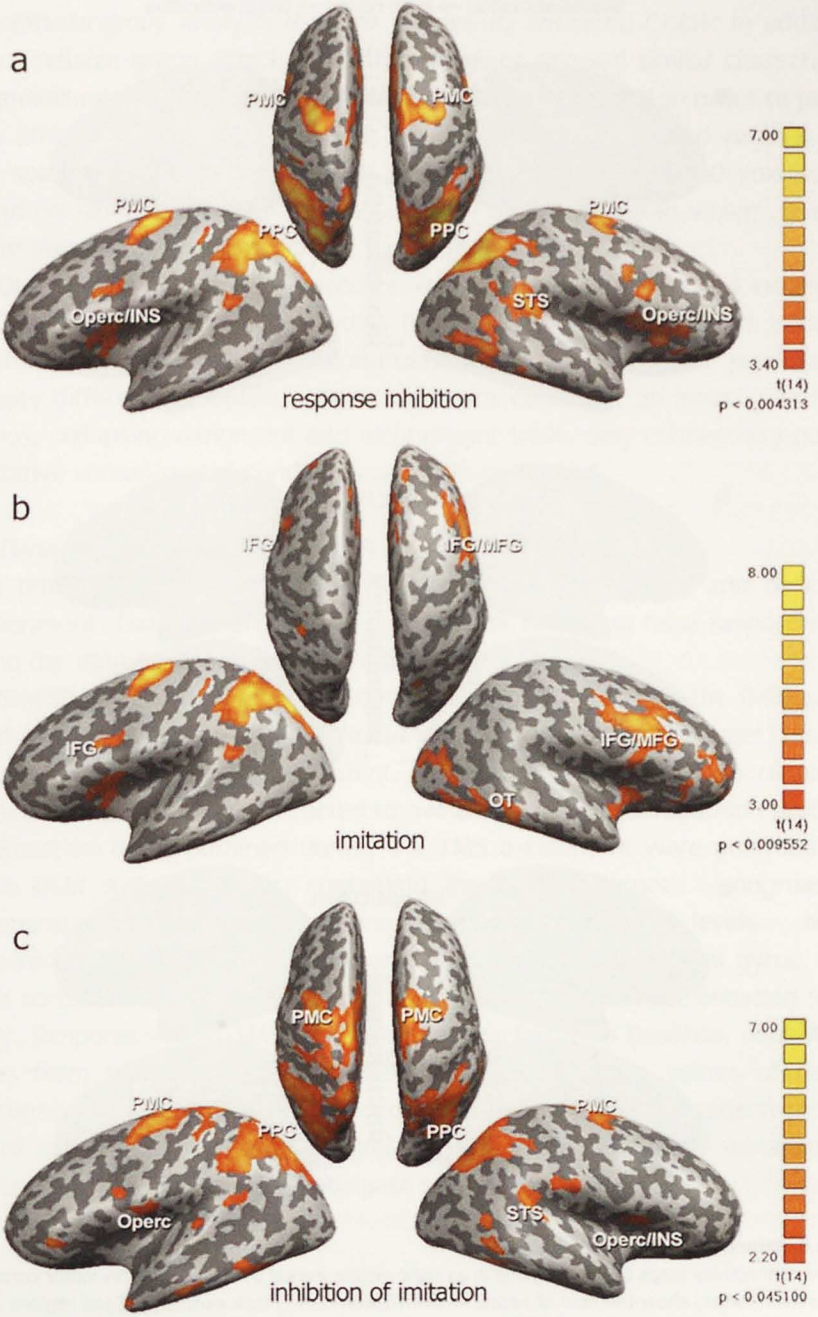

Figure 3. fMRI results
Random effects GLM activation maps projected onto inflated MNI cortical surfaces. Cluster size treshold was set at 50, and maps were smoothed in one iteration. a) Response inhibition, reflected by incongruency main effects, contrast: incongruent – congruent. b) Imitation main effects, contrast: imitative – spatial. c) The inhibition of imitative responses: spatial incongruent – the other three main experimental conditions.

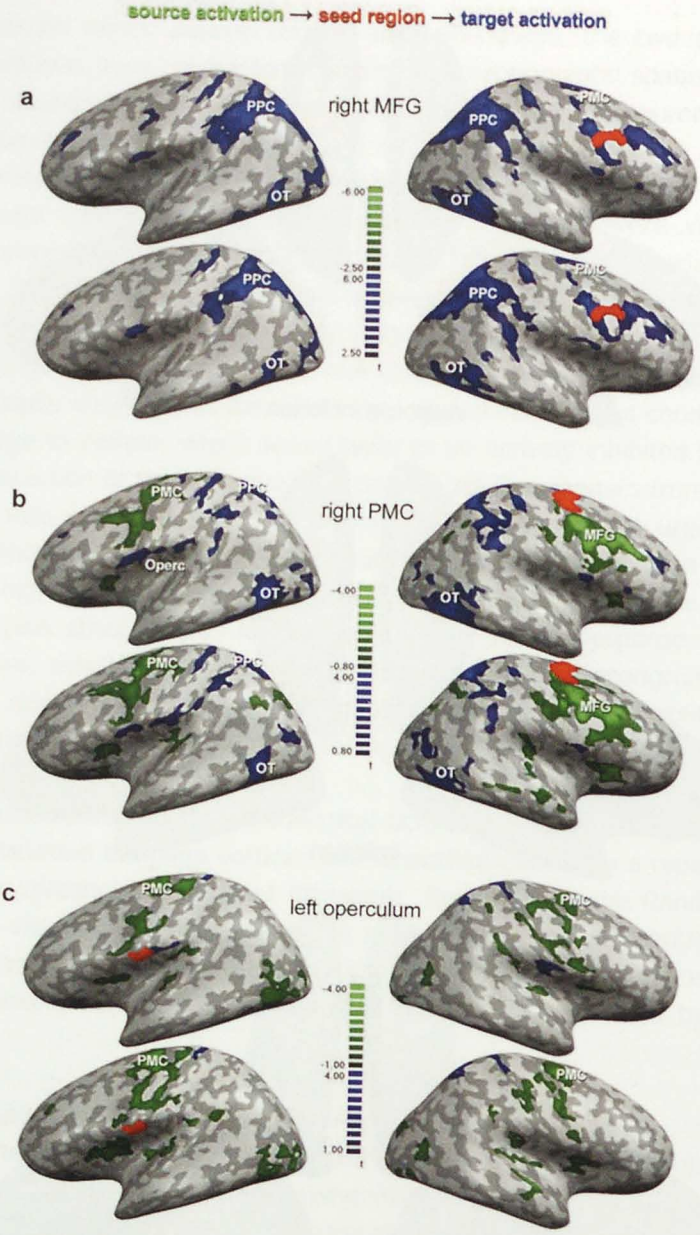

Figure 4. Effective connectivity maps

Effective connectivity maps of seed regions in a) right middle frontal gyrus, b) right premotor cortex, and c) left opercular cortex, show the flow of neuronal information during task execution. Seed regions are shown in red, green colours display regions serving as an activation source of the seed region, and blue colours represent target regions, receiving neural input from the seed region. Maps obtained from imitative blocks are shown in the top row of each panel, maps obtained from spatial conditions are in the bottom row of each panel. Right middle frontal gyrus (response inhibition) projects to right premotor cortex (imitation), which in turn projects to posterior parietal cortex (which possibly sends feedback) and finally to left opercular cortex (inhibition of imitation).

Random Effects group analysis (GLM or an already executed GCM). In addition to showing a reliable group effect, all of these regions showed similar characteristics in each individual subject. The latter prerequisite has to be met in order to justify a Random Effects GCM, which demands individually localized seed regions. Each selected seed region was chosen such as to consist of 200 to 300 voxels; large enough to consider it a representative selection of the activated voxels, but small enough to prevent heterogeneity from concealing the results.

Due to the mixed, randomized presentation of congruent and incongruent trials –a decision based on pilot studies that indicated that a blocked, separated presentation of congruent and incongruent trials would be too predictable – connectivity differences between these conditions could not be investigated using GCM. Thus, collapsing congruent and incongruent trials, only connectivity patterns from imitative versus spatial conditions could be compared.

Behavioural and TMS data analysis

Reaction times were recorded during the brain imaging session and during the TMS experiment. Data pre-processing consisted of removing false responses, and inspecting the data for skewness and outliers.

Statistical analysis of the reaction times recording during the fMRI session consisted of a Repeated Measures ANOVA containing the factors 'target' (imitative or spatial) and 'congruency' (congruent, incongruent). Alpha values of pairwise comparisons were Bonferroni corrected to avoid the multiple comparison problem.

Reaction times obtained during the TMS experiment were analysed using univariate GLM statistics, again containing the factors 'target', 'congruency' – either separately, or collapsed into one factor 'condition' of 4 levels -, and an added factor 'target site' (right premotor cortex, right middle frontal gyrus, or left opercular cortex). A factor 'subject' was added to compensate for between subject variability. Responses from TMS trials were subtracted from baseline, consisting of responses from trials in which no TMS was applied. Alpha values of pairwise comparisons were LSD corrected with regard to the multiple comparison problem. In case of polynomial factors the degrees of freedom of the error variance were Greenhouse-Geisser corrected to anticipate sphericity issues.

Results

Behavioural results obtained during the brain imaging experiment

The 2 by 2 repeated measures analysis of all 15 fMRI participants over all the experimental conditions yielded significant main effects for both of the included

factors. With regard to the factor target ($P = 0.026$), subjects responded significantly faster to spatial (mean RT 432.8 ms, SD 17.3 ms) than to imitative trials (mean RT = 442.8 ms, SD = 16.8 ms). Also the factor congruency showed a significant main effect ($P < 0.001$), in that subjects responded considerably slower in the incongruent condition (mean RT 466.2 ms, SD 19.4 ms) compared to the congruent condition (mean RT 409.5 ms, SD 15.1 ms). No significant interactions were found.

Brain imaging results

Regions supposedly reflecting response inhibition were revealed by showing the incongruency main effect, obtained by contrasting incongruent with congruent trials (Figure 3a). This contrast revealed activations in the premotor cortex and middle frontal gyrus (dorsolateral prefrontal cortex), especially in the right hemisphere, intraparietal sulcus and inferior parietal gyrus bilaterally, right anterior cingulate cortex (ACC), right pre-SMA, right STS, and the anterior insula / frontal operculum, again slightly lateralized to the right. Although there is evidence that cortical inhibition correlates with negative BOLD changes, we did not find such patterns in the current data. A few negative clusters emerged from the analysis, however closer inspection of their event-related deconvolutions showed that they were most likely the results of artefacts, not functional de-activations.

Brain regions showing an increased BOLD response to imitative relative to spatial trials consisted of the posterior part of the right inferior frontal gyrus, and the occipitotemporal cortex (Figure 3b). The activation was bilateral, although much more pronounced in the right hemisphere. No brain areas were found that showed a stronger response to spatial than to imitative cues.

The contrast hypothesized to reveal regions specifically involved in the inhibition of automatic imitation, namely spatial incongruent versus all other three main experimental conditions, showed an extensive network, comprising premotor cortex, posterior parietal, and frontal / parietal opercular cortex, all bilateral, and right STS (Figure 3c). However, only few of these regions actually showed a BOLD response that was specifically increased during spatial incongruent trials. Only the left opercular region showed a clear BOLD response depicting a stronger activation in the spatial incongruent condition compared to the other three experimental conditions.

Functional and effective connectivity results

In order to reveal the direction of information flow within the revealed network, effective connectivity analyses were conducted using Granger Causality Mapping (GCM, (Roebroeck et al., 2005)), producing cortical maps depicting functional and

effective connectivity, consisting of source and target activations. Source activations represent regions whose activationconsistently preceded that of the seed region, and thus are hypothesized to have exerted task-related causal influence on the cortical activation of the seed region. Target activations on the other hand represent regions that are consistently activated after the seed region, and thus are hypothesized to be causally influenced by the seed region.

As starting points for effective connectivity, three seed regions were used, whose selection was based on the previously described brain imaging results. The first region of interest was located in right premotor cortex. It displayed a consistent incongruency effect in the overall GLM as well as in all 15 individual subjects, and also showed corresponding event-related deconvolutions in each subject. The second region of interest used as a seed for effective connectivity analysis was produced by the connectivity analysis of right premotor cortex as an area sending neuronal input, and was located in the right middle frontal gyrus. The third seed region was located in the left opercular cortex, and displayed a preference for spatial incongruent trials, and was thus hypothesized to be crucial to the inhibition of imitative responses.

Effective connectivity analyses revealed that right premotor cortex receives neural input from predominantly right middle frontal gyrus and frontal opercular / insular regions, and in turn projects neural input to bilateral posterior parietal, frontoparietal opercular and occipitotemporal cortical target regions (Figure 4b). These effective connectivity networks were similar in imitative and spatial blocks, and thus in accordance with the hypothesis, based on this regions BOLD responses, that this network is involved in a general incongruency effect - probably reflecting response inhibition- in both imitative and spatial blocks. As a whole, and considering the heightened BOLD responses of the right premotor region during incongruent trials, this suggests that the network is involved in response inhibition, and displays a flow of information from middle frontal and insular regions, via premotor cortex, to bilateral posterior parietal, frontoparietal opercular and occipitotemporal cortices.

The same analysis was carried out over the right middle frontal gyrus, which was consistently present as a source of information for the activation in right premotor cortex. This confirmed the flow of neuronal information from right middle frontal to right premotor cortex (Figure 4a). Furthermore, posterior parietal, opercular and occipitotemporal cortex were shown to be targets of right middle frontal gyrus. Since GCM cannot distinguish between influence exerted directly or via an intermediate cortical area, these target activation probably reflect the aforementioned target activations of right premotor cortex, which thus functions as a mediator. Again, few differences were observed between imitative and spatial

conditions. The presence of many cortical areas receiving information from middle frontal cortex, whereas almost none are sending information, indicates a relatively early timepoint of relevance within the activated network.

Effective connectivity analysis over the left opercular seed region, according to its BOLD pattern specifically involved in suppressing the urge to imitate, once more showed no large difference between imitative and spatial blocks, although the source activation in insular / opercular regions was slightly more pronounced in the spatial condition (Figure 4c). In this case, compared to the right premotor and moreover the right middle frontal connectivity maps, much more source activations were observed, implying that this area was activated in a later stage than the right premotor and middle frontal areas. Among the source regions were areas along the precentral gyrus and sulcus, anterior insular / frontal opercular regions, and occipitotemporal cortex, bilaterally. It is important to note that the aforementioned right premotor seed region, earlier seen to project information to left opercular cortex, was also included in these areas, confirming that its activation served as a source of information for the left opercular region.

Taken together, these results suggest that, assuming the left opercular region is indeed involved in the inhibition of imitation as its BOLD pattern suggests, it mainly receives its information from insular/opercular, occipitotemporal, and frontal regions, the latter including the middle frontal area, which is sending input to the right premotor area.

TMS results

The three selected target regions consisted of the right premotor area that displayed an incongruency effect and was also used as a seed region for effective connectivity analysis, a right middle frontal region that was revealed by effective connectivity analysis as a source region of the aforementioned TMS target region, and the left opercular region that displayed a strong activation as a result of spatial incongruent trials (Figure 5). Although the activation of premotor cortex was largely bilateral, right premotor cortex was chosen to prevent direct influence of TMS on motor performance during task execution. Furthermore, the selection criteria ruled out several interesting candidate regions in the parietal lobe, due to a very high degree of intersubject variability.

An overall view of the results showed that when neural activity in any of these regions was disrupted by TMS, participants on average responded significantly faster as compared to non-TMS blocks ($F = 135.9$, $df = 1$, $P < 0.001$) (Figure 6). This effect was seen following stimulation of any of the three target regions, and was thus non task- or area-specific. However, an overall GLM consisting of the factors region (3 levels) and condition (4 levels) yielded a signifi-

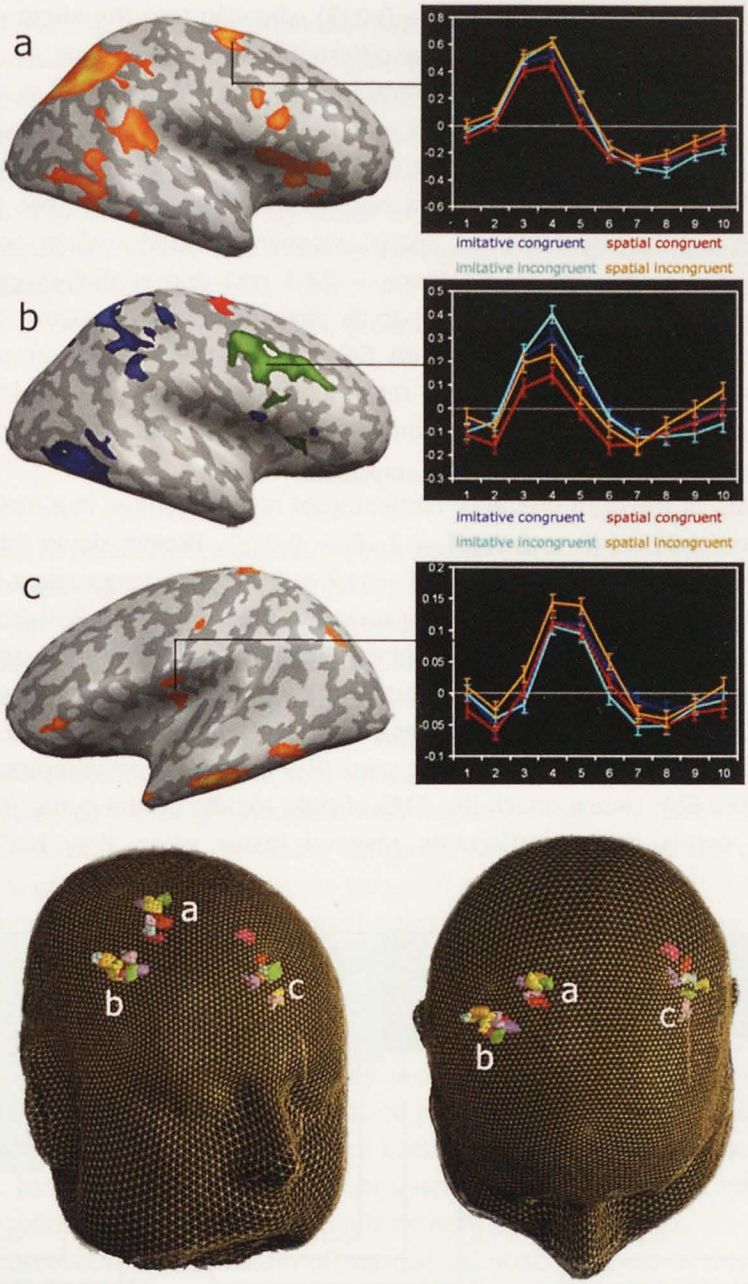

Figure 5. TMS target regions

The three target regions for the TMS experiment, visualized on an inflated MNI hemisphere (top panel) and as clusters in a 3D reconstruction of the head, color coded per subject (bottom panel). a) Right middle frontal gyrus was produced consistently as a source of the cortical activation in right premotor cortex (shown in red) in effective connectivity maps. b) Right premotor cortex showed an increased activation during incongruent relative to congruent stimuli, as shown by its event-related deconvolution. c) A region in the left operculum shows a preference for spatial incongruent trials, the condition presumed to reflect inhibition of imitation, as shown in orange in the event-related deconvolution.

cant interaction ($F = 2.4$, $df = 6$, $P = 0.027$), showing that the effect of condition was significantly different for the three different TMS target regions.

Disturbing the right middle frontal gyrus with TMS resulted in a significant main effect of condition ($F = 4.75$, $df = 3$, $P = 0.003$), and when broken down, in significant main effects of both target ($F = 4.2$, $df = 1$, $P = 0.041$) and congruency ($F = 7.4$, $df = 1$, $P = 0.007$). Post hoc comparisons showed that participants responded significantly faster to spatial incongruent trials, when compared to imitative ($P = 0.001$, mean difference = 23.4 ms) and spatial congruent ($P = 0.003$, mean difference = 20.4 ms) trials, but not when compared to imitative incongruent trials ($P = 0.147$; Figure 6a). Thus, after stimulation of the right middle frontal cortex participants responded faster to trials which required inhibition of automatic imitation, compared to when their right middle frontal cortex was not disturbed by TMS.

TMS of the right premotor cortex target region resulted in a main effect for the factor condition ($F = 3.2$, $df = 3$, $P = 0.022$). Broken down into separate factors, the factors target ($F = 3.5$, $df = 1$, $P = 0.062$) and congruency ($F = 3.7$, $df = 1$, $P = 0.056$) showed trends towards main effects. Post-hoc pairwise comparisons between the four different conditions showed that subjects responded significantly faster in the spatial incongruent condition than to imitative congruent ($P = 0.005$, mean difference 21.4 ms), imitative incongruent ($P = 0.014$, mean difference 18.6 ms), and spatial congruent ($P = 0.017$, mean difference 18.0 ms) trials (Figure 6b). Hence, much like TMS of right middle frontal gyrus, TMS of right premotor cortex made participants respond faster when they had to inhibit

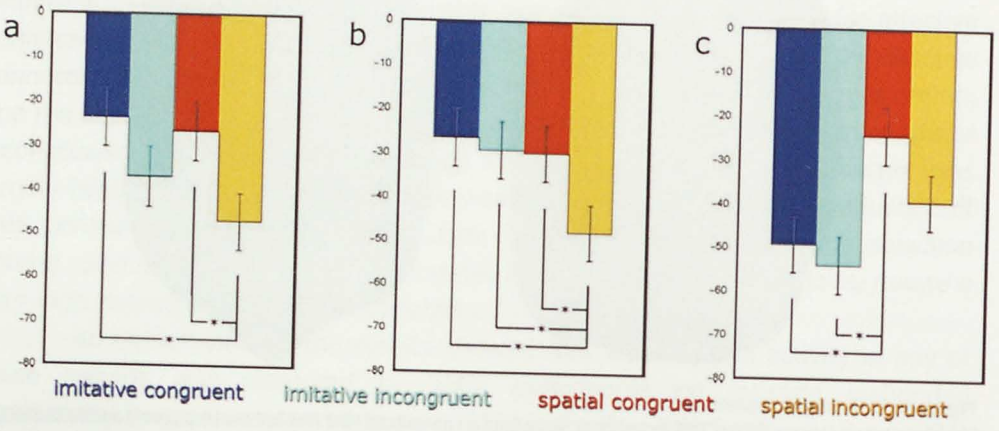

Figure 6. TMS results

Condition-averaged reaction times (ms) of TMS – baseline (no TMS), shown for each target region: a) right middle frontal gyrus, b) right premotor cortex, and c) left frontoparietal operculum. Asterisks represent comparisons significant at the LSD-corrected α=5% level.

automatic imitation, relative to when their right premotor cortex was not disrupted.

Administering TMS to the left opercular target region again resulted in a main effect of condition ($F = 5.0$, $df = 3$, $P = 0.002$). Broken down, only the factor target revealed a significant main effect ($F = 11.5$, $df = 1$, $P = 0.001$). Post hoc pairwise comparisons showed that compared to spatial congruent trials, participants responded significantly faster in the imitative congruent ($P = 0.014$, mean difference = 25.0 ms) and imitative incongruent ($P = 0.002$, mean difference = 29.7 ms) conditions (Figure 6c). Thus, when left opercular cortex was disturbed, participants responded relatively faster in conditions requiring intentional imitation.

Discussion

Our goal was to reveal the existence, layout, connectivity, and functional relevance of the brain network enabling us to inhibit automatic imitation. In order to do this, and based on previous brain imaging studies successfully employing the same design (Iacoboni et al., 1999), we used the stimulus-response paradigm to map the human mirror neuron system, and compare different forms of cortical inhibition, including the sought after inhibition of imitation. Using different complementary methods we were able to not only observe, but also experimentally manipulate the brain activity associated with response inhibition. This resulted in a new neurobiological model explaining the processes and mechanisms underlying the inhibition of imitative responses.

Conditions reflecting a general stimulus-response incongruency effect showed a slightly right-lateralized cortical network consisting mainly of premotor cortex, operculum/insula, posterior and intraparietal cortex, and middle/inferior frontal gyrus. This latter cortical area was predominantly involved in the processing of imitative stimuli. Effective connectivity analysis showed a flow of information through this network from middle frontal, to premotor, to parietal cortex. Possibly feedback was sent from parietal cortex back to the premotor cortex. The left operculum, according to its BOLD pattern specifically involved in the inhibition of imitative responses, received information from right premotor cortex.

To unravel the contribution of the cortical areas involved in this network, we targeted three regions with TMS, enabling us to observe the causal relevance of these regions for the behaviourally controlled inhibition of imitative responses. TMS induced a general and consistent speeding of response times. As a consequence, it is difficult to say whether a shorter reaction time in a certain condition is indeed a sign of improved performance, or whether it reflects

deteriorated performance in the other conditions. However, the issue with regard to whether a change in performance was in absolute sense positive or negative can never be ruled out, and does not interfere with drawing conclusions about the functional relevance of, in this case, brain areas targeted with TMS. Interestingly, and aside from this neither task- nor area-specific behavioural improvement effect, there were also task- and area-specific effects, as could already be concluded from the significant interaction between TMS target region and task condition.

Disturbing right premotor cortex resulted in relatively faster responses in the only condition during which an automatic imitative response would not be beneficial, even counterproductive: the spatial incongruent condition. We conclude from this that premotor cortex is involved in the process of automatic imitation, and that disrupting premotor activity with TMS inhibited this tendency of automatic imitation, thereby resolving the incongruency effect resulting from competition between the spatial and the imitative stimulus. In other words, when the urge to imitate the "wrong" finger movement is reduced by TMS-induced premotor activity disruption, moving the "correct" finger as indicated by the spatial target stimulus becomes easier, hence faster.

Disturbing right middle frontal gyrus during task execution resulted in a pattern similar to that caused by disturbing the right premotor cortex, a significant speeding up of especially the responses to spatial incongruent trials. This behaviourally supports the results of the effective connectivity analysis, which suggests that right premotor and middle frontal cortex are, with regard to response inhibition, nodes of the same functional network. However, we did observe a difference between TMS of the right premotor versus middle frontal areas: in case of the latter, the difference between spatial and imitative incongruent conditions is not significant. This implies that, whereas premotor cortex serves to process imitative stimuli as discussed earlier, the target area in middle/inferior frontal cortex is possibly concerned with response inhibition in general. This conclusion seems in accordance with the findings of Brass and colleagues (Brass et al., 2005), who reported a similar cluster showing mutual activation in two inhibition tasks, which they interpreted as an area generating a final stop signal concluding different instances of response inhibition. However, our combined evidence suggests a functional role for the inferior/middle frontal region fairly early in the process, rather than at the very end. The postulation is on the other hand challenged by the GLM results, which show involvement of premotor cortex in response inhibition, and of middle frontal gyrus in the processing of imitative stimuli. Although interesting, this debate currently remains speculative since it is not backed up by statistical significance.

Stimulation of the left opercular region, which showed an increased BOLD in response to the condition during which the inhibition of imitation was of utmost importance, resulted in speeded response times in the imitative conditions, especially compared to the spatial congruent condition. It is important to note that during the spatial and imitative conditions the visual display is entirely the same, and that during the congruent conditions even the correct response is identical, since both cues are always presented on the same finger. In this light, the observed difference between the imitative conditions and the spatial congruent condition is remarkable. The only difference between these conditions is that in the former the observed actions are intentionally imitated, whereas in the latter case they are unintentionally imitated. In addition, effective connectivity analyses showed that this area within the left opercular region receives information from middle frontal cortex (which according to our findings is involved in response inhibition) through right premotor cortex (involved in imitation), which implies a seemingly late timepoint of relevance in the cortical network. We therefore suggest that the left frontoparietal operculum is involved in intentional imitation, and serves as a final gating mechanism deciding on whether to execute the imitative action prepared by premotor cortex, or not. Thus, whereas automatic imitation is the unconsciously initiated imitative response of mirror neurons to the sensory perception of biological motion, intentional imitation occurs when a person consciously decides to imitate an observed movement. The mirror neuron system is most likely involved in both, and there is probably considerable continuity between automatic and intentional imitation. They share the first stages of the process, and in the case of intentional imitation, inhibition is released at the final stage of the procedure.

The concept of an inhibitive component serving as a final gating mechanism on an already prepared motor response may come across as counterintuitive and inefficient. However, one must keep in mind that this prepared imitative motor action is a result of the activation of the (pre)motor areas in response to action observation, hence in essence an epiphenomenon of action understanding and the deriving higher order cognitive processes associated with the mirror neuron system. Furthermore, a similar concept of a gating mechanism inhibiting an already prepared motor response was also brought up by Brass and colleagues (Brass et al., 2005).

Subtracting spatial from imitative trials was expected to reveal areas usually associated with the mirror neuron system, especially Broca's area (left inferior frontal gyrus and frontal operculum), left premotor cortex, and parietal cortex. In this light, the lack of activation in Broca's area was unusual, contradicting amongst others the evidence described by Heiser and colleagues

(Heiser et al., 2003), who concluded that this brain region is crucial for imitation. It has been shown that even static images conveying motion can induce cortical activation usually related to action observation (Nishitani & Hari, 2002). In the current experiment this would be the case, even increasingly so as time during the experiments elapsed and participants got more and more acquainted with the stimulus and its relation to motion. Preliminary activation would prevent mirror neuron related areas from appearing in activation maps.

Thus, with regard to the intended mapping of the mirror neuron system, we conclude that the employed stimulus-response compatibility paradigm did not yield the typical set of cortical areas usually associated with mirror neuron activity. Neither did we find the reaction time advantage in response to imitative stimuli, again contrarily to the expectance based on previous studies using the same paradigm. Both findings are, however, in accordance with several other studies not finding mirror neuron related cortical activity as a result of action observation (Jackson, Meltzoff, & Decety, 2006; Makuuchi, 2005), as well as a recent study by Jonas and colleagues (Jonas, Siebner, Biermann-Ruben et al., 2007), putting forward the notion that, depending on the experimental context, the stimulus-response compatibility task does not always reliably activate the mirror neuron system. Following this reasoning, we propose that in the experimental context of the brain imaging part of our study, the stimulus-response compatibility task might have encouraged participants to, to a certain extent, follow a spatial matching strategy besides employing their mirror neuron system. In conclusion, our setup

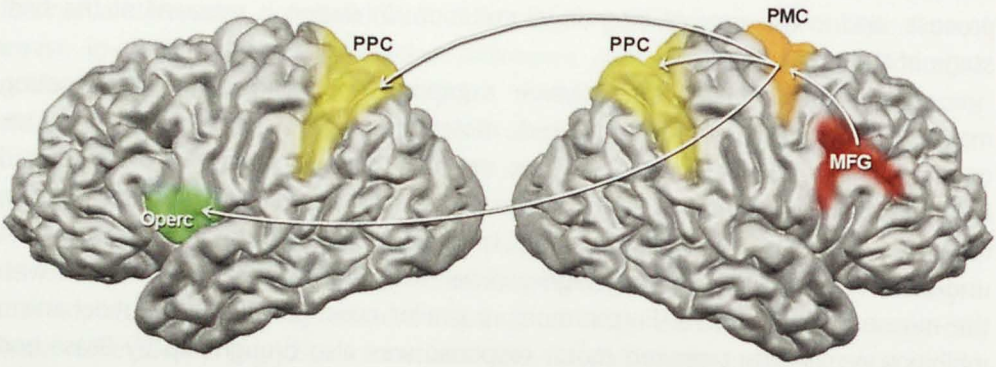

Figure 7. Neurobiological model
A neurobiological model for the inhibition of automatic imitative responses, as proposed by our findings. Right middle/inferior frontal cortex (red) sends neural input concerning response inhibition to premotor cortex (orange), which is involved in the process of automatic imitation. Subsequently, information is sent to bilateral posterior parietal and intraparietal cortices (yellow), which potentially return feedback to premotor cortex. Finally, left opercular cortex (green) receives information from premotor cortex, and exerts its function in the specific inhibition of automatic imitation, serving as a final gating mechanism for intentional imitation. These findings suggest a functional dissociation between automatic and intentional imitation, a distinction supported by neuropsychological literature.

was not ideal for mapping the mirror neuron system. This was however not our primary goal, and does not necessarily interfere with the gist of this study.

In this study several methods were employed, each with their own strengths and weaknesses. Combining these methods in a way that they become complementary to each other resulted in a deepened understanding of observed brain activations, which could not have been obtained using any of these methods in isolation. In conclusion, our evidence converges to a new neurocognitive model for the mechanisms and cortical components associated with the inhibition of automatic imitative responses (Figure 7). Information concerning general incongruency and response inhibition flows from right middle and inferior frontal cortex to a right premotor region involved in imitative responses. Subsequently, information is sent to the posterior parietal and intraparietal cortices, and possibly sent back in the form of feedback to the premotor cortex. Finally, information is sent from right premotor cortex to left frontoparietal operculum, which subsequently acts upon this information as a final gating mechanism deciding on intentional imitation, reflecting the inhibition of an already prepared imitative response.

Considering the previously described neuropsychological reports by Luria and Lhermitte concerning patients exhibiting increased imitation behaviour after having sustained frontal lobe damage, it seems unexpected to conclude that the inhibition of imitation would be settled in this somewhat differently located cortical area. On the other hand, the participation of the middle and inferior frontal lobe in response inhibition in general has since long been established (Dillon et al., 2007; Harrison et al., 2005; Horn et al., 2003; Langenecker et al., 2004; Milham et al., 2002; Vendrell et al., 1995; Wager et al., 2005), and is supported once more by the current results. With regard to the specific inhibition of imitative responses, the quantity of neuropsychological literature is rather limited, and inconclusive with regard to the exact neurobiological mechanisms underlying this process.

We propose that, besides the premotor cortex which is involved in automatic imitation, and the middle frontal cortex which subserves general response inhibition, the described frontoparietal area is part of the cortical network enabling healthy human beings to imitate in an intentional manner, and to inhibit their already planned imitative responses when needed. This functional neural dissociation between automatic and intentional behaviour as revealed by our study, is also supported by the neuropsychological distinction, as proposed by Lhermitte (Lhermitte et al., 1986), between the involuntary, reflex-like form of imitation associated with echopraxia, and the voluntary, complex imitation behaviour associated with obstinate imitation behaviour. According to our neurobiological model, patients suffering from echopraxia or imitation behaviour

may have sustained defects at one or several levels of this network, either resulting in increased imitation, or decreased inhibition of imitation, or a combination of both.

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Chapter 3

Extinguishing extinction

Hemispheric differences in the modulation of TMS-induced visual extinction by directing covert spatial attention

Based on:
Bien, N., Goebel, R., and Sack, A.T. (submitted). Extinguishing extinction. Hemispheric differences in the modulation of TMS-induced visual extinction by directing covert spatial attention.

Abstract

The topic of spatial attention is of great relevance for researchers in various fields, including neuropsychology, cognitive neuroscience, and cognitive psychology, as well as for clinical practice. Deficits of spatial attentional arising from parietal brain damage remain largely confined to the left visual field. The mechanisms underlying this hemispheric asymmetry are still elusive. We mimicked the neuropsychological syndrome of contralesional extinction by temporarily inducing a spatial attentional bias in healthy volunteers with TMS. We investigated whether directing covert spatial attention could enhance or, more importantly, counteract the resulting behavioural deficits. Although both left and right parietal TMS induced contralateral extinction, only left hemifield extinction following right parietal TMS was severely aggravated by a competing stimulus in the ipsilesional (right) hemifield. We put forward the hypothesis that an asymmetry with respect to the ability of detaching attention from a distractor is contributing to the right hemispheric lateralisation with regard to extinction. On a broader level, we suggest that "virtual patients" might be used for evaluating neuropsychological treatment in an early stage of development, reducing the burden on actual patients.

Introduction

Damage to parietal cortex often results in spatial attention deficits, the most prominent of which is known as hemispatial neglect. Patients suffering from hemispatial neglect fail to perceive and react to stimuli appearing in the contralesional hemifield, and have been shown to display increased perception and anticipation towards stimuli appearing in the ipsilesional hemifield (see e.g. Kerkhoff, 2001; Vallar & Perani, 1986). In healthy adults, commonly a slight attentional bias towards the left hemifield is observed, a phenomenon referred to as pseudoneglect (Orr & Nicholls, 2005). This phenomenon, resulting for example in overestimation of the left part of a line in a line bisection task, indicates a dominance of the right hemisphere over the left hemisphere in healthy right handed adults. The neuropsychological syndrome of hemispatial neglect, on the other hand, is strongly lateralised in the opposite direction: it is most frequently observed after right hemispherical damage, and thus usually induces hemispatial neglect of the left and an attentional bias towards the right hemifield (Vallar et al., 1986). A condition often bracketed together with hemispatial neglect is extinction. Patients suffering from extinction also fail to report a stimulus in the contralesional hemifield, but only if this stimulus is presented simultaneously with a competing stimulus in the ipsilesional hemifield. This failure to detect bilateral stimuli increases with stimulus eccentricity (Smania, Martini, Gambina et al., 1998). Extinction in response to bilateral visual stimuli has also been demonstrated in healthy adults, as a result of saliency manipulation of the stimulus in the contralateral hemifield (Gorea & Sagi, 2000; Meador, Ray, Day et al., 1998).

It is still highly debated whether or not neglect and extinction are part of the same neuropsychological condition. Nonetheless, the terms are often used interchangeably (Corbetta & Shulman, 2002; Hilgetag, Theoret, & Pascual-Leone, 2001; Manes, Paradiso, Springer et al., 1999), and extinction is sometimes referred to as a milder form of neglect (Hilgetag et al., 2001). Often spatial neglect is very broadly defined, like "disorders of spatial cognition which concern a specific sector of space with reference to a given coordinate system" (Vallar, Rusconi, & Bernardini, 1996). However, there is increasing evidence that neglect and extinction may represent two different neuropsychological conditions, and respective double dissociations between neglect and extinction are common (Becker & Karnath, 2007). Pavlovskaya (2007) found extinction in neglect patients even after they controlled for the difference in attentional saliency between the affected and healthy hemifield. It has also been proposed that whereas neglect is largely confined to right hemispherical damage, extinction is much less subject to

hemispherical asymmetry (Becker et al., 2007; Bonato, Priftis, Marenzi et al., 2010).

At present, two competing theories try to account for the highly asymmetrical manifestation of hemineglect. Since it can be assumed that lesions are as likely to occur in the left hemisphere as they are in the right, the answer must lie in the asymmetry of the expression of these lesions rather than in the location of their occurrence. The finding that neglect is most common following right-sided lesions has led many to assume a hemispheric asymmetry in spatial attention (Corbetta, Miezin, Shulman, & Petersen, 1993). It is proposed that the left hemisphere controls attention only in the right hemifield, whereas the right hemisphere is able to control both sides (Corbetta et al., 1993; Heilman, Bowers, Valenstein, & Watson, 1993; Mesulam, 1981). This implies that the right hemisphere is able to compensate for a lesion in the left hemisphere, but not vice versa (Sack, Camprodon, Pascual-Leone, & Goebel, 2005; Sack, Kohler, Bestmann et al., 2007; Sack, Sperling, Prvulovic et al., 2002). These empirical accounts and clinical phenomena are formalised in the theory of *hemispheric asymmetry in attention*, with the right hemisphere being dominant (Corbetta et al., 1993). An alternative model of neglect, the *opponent-processor model*, has been proposed by Kinsbourne (1993), claiming that the attention of each hemisphere is biased in the direction of the contralateral hemifield, with the rightward bias of the left hemisphere being stronger. Normally, both hemispheres mutually inhibit each other's orienting biases, leading to equal allocation of spatial attention across space. This balanced gradient remains until one of the two hemispheres is damaged. If this occurs, the inhibition of the intact hemisphere by the injured hemisphere is reduced and it becomes overactivated, thereby further suppressing function in the injured hemisphere. This results in a spatial deficit contralateral to the lesion. Since the contralateral orientation bias of the left hemisphere is stronger, its reduced suppression after right parietal damage results in more pronounced behavioural deficits, as documented by the higher frequency of hemispatial neglect of the left and an attentional bias towards the right hemifield.

Hence, while claiming different underlying physiological mechanisms, both theories account in their own way for the fact that the prevalence of neglect is much higher following right hemisphere lesions. This makes it difficult to distinguish between the different models at a behavioural level. Since spatial attention deficits such as hemispatial neglect and extinction normally only become manifest after brain damage, their underlying mechanisms have almost exclusively been studied in lesion patients. The reasons for studying patients are twofold. On the one hand, by understanding which changes occur in brain and behaviour, and by assessing how patients respond to medication or other rehabilitative

treatments, it is hoped that the functional damage can be reduced or, optimally, even reversed. On the other hand, for this venture to be successful, knowledge of the mechanisms underlying certain functionality in the healthy brain is crucial. By evaluating which cognitive changes occur in lesion patients compared to healthy adults, a vast amount of information can be obtained about functions previously represented in the now damaged part of the brain, like directing spatial attention.

However, investigating the cognitive consequences of brain lesions is hampered by several limitations. Firstly, lesions are rarely small enough to be confined to a single functional area or module in the brain, which means that their resulting deficiencies often pervade into multiple functional domains. This makes it difficult to ascribe certain functions to specific parts of the brain. Secondly, the cognitive consequences of a lesion are not stable over time. Especially in the first days and weeks following lesion onset, the brain goes through intense processes of plastic changes, functionally reorganising itself, trying to compensate for the damage and maintain as much functionality as possible. As a result of this cortical plasticity, the functional layout of the brain is changed, which could lead to an underestimation (or even overestimation in case of maladaptive plasticity) of the relevance of a certain brain area for a given function in the healthy brain (Sack, 2010). Thirdly, no two lesions are exactly the same with regard to their location, size and functional impact, which further distorts comparisons between lesions and their functional consequences. Fourthly, patients suffering from lesion-induced loss of brain function are in the majority of the cases only monitored from the moment they report their deficits and seek treatment. This makes it impossible to objectively compare cognitive brain functioning after the lesion to cognitive functioning in the same person before lesion occurrence, when the brain was presumably still healthy. Finally, in the same line of thought, differences in age, education, personality, and other possibly confounding background factors cannot be controlled for, for the simple reason that patients can by definition not be randomly assigned to a patient or non-patient group.

Despite these limitations, studying patients suffering from altered cognitive functioning as a result of brain damage has so far been an extremely important source of information about the functional architecture of the healthy brain. Some of the limitations mentioned above can partially be counteracted by taking measures such as investigating only patients in the acute state of a lesion, and by comparing patient characteristics to those of healthy controls, which are preferably matched to the patient group with regard to most confounding criteria such as age and educational level.

Nevertheless, a more elegant way to investigate the relationship between brain and behaviour under controlled experimental conditions, and to study brain

functions of neurologically healthy, randomly selected adults who can serve as their own controls when comparing behavioural performance with and without local neural activity disruptions, is transcranial magnetic stimulation (TMS). Unlike functional imaging which provides observational measures of the functional architecture of the healthy or diseased brain, TMS allows for non-invasive direct local interference of cortical processing in a certain brain area, thus temporarily creating a 'virtual lesion' in conscious healthy volunteers. Observation of resulting behavioural changes provides causal information about the functional relevance of a certain brain area. With this virtual-lesion approach of TMS, neuropsychological conditions can temporarily be mimicked in healthy volunteers under controlled experimental conditions (Sack, 2010). This allows for random selection of 'patients', or balanced selection with regard to a certain trade, if relevant. It also allows for within-person balanced comparison between behaviour observed with and without the virtual lesion, ruling out the many confounding factors that limit most patient studies.

This TMS virtual lesion approach has successfully been applied to the domain of visuospatial attention. Pascual-Leone and colleagues (1994) already observed that parietal repetitive TMS applied to healthy adults led to a large number of misses of the contralateral part of a bilateral stimulus, whereas performance on unilateral trials remained unaffected by TMS. Fierro and colleagues (2000) aimed to mimic hemispatial neglect in healthy volunteers. After right, but not left, parietal TMS an overestimation of the length of the right section of a bisected line was observed, indicating a rightward shift in attentional bias. By now, several studies have demonstrated that parietal TMS is capable of causing contralateral hemineglect in healthy volunteers (Bjoertomt, Cowey, & Walsh, 2002; Dambeck, Sparing, Meister et al., 2006; Fierro, Brighina, & Bisiach, 2006; Fierro et al., 2000; Fierro, Brighina, Piazza et al., 2001; Hilgetag et al., 2001; Koch, Oliveri, Cheeran et al., 2008; Koch, Oliveri, Torriero, & Caltagirone, 2005; Meister, Wienemann, Buelte et al., 2006; Muggleton, Postma, Moutsopoulou et al., 2006; Pascual-Leone et al., 1994). Taken together, these studies showed that TMS can be employed to mimic lesions under controlled experimental conditions, in healthy adults (Sack, 2010).

In the same line, Hilgetag and colleagues (2001) applied 10 minutes of 1 Hz repetitive TMS to the right or left intraparietal sulcus (P3 and P4 of the 10-20 EEG positioning system, respectively) during the execution of a visuospatial detection task. In addition to virtual neglect, they also observed virtual extinction. Healthy volunteers showed decreased detection of the contralesional part of a bilateral stimulus, an effect that was most strongly observed in the left hemifield, as a result of right parietal TMS. Interestingly, Hilgetag et al. (2001) also reported

an increase in detection of unilateral stimuli appearing in the periphery of the ipsilesional hemifield. In line with evidence from real lesions, this occurred most strongly in the right hemifield, after right parietal TMS. According to Kinsbourne's opponent-processor model (1993) hemispatial neglect is a result of a disturbance in interhemispheric balance, which causes the attentional gradient to shift. The authors thus interpreted their ipsilesional enhancement finding as a consequence of a reduced suppression of the left hemisphere by the TMS-disrupted right hemisphere. However, theoretically and consequently in line with this logic, a simultaneous lesion in both parietal cortices should restore this balance and result in unaffected visuospatial attention. This scenario was experimentally created by Dambeck and colleagues (2006) using the virtual lesion approach of TMS. First, the virtual neglect and extinction reported by Hilgetag (2001) were replicated by applying single pulse TMS over the right parietal hemisphere, 150 ms after stimulus onset. Subsequently, TMS was applied simultaneously over the previously unstimulated left hemisphere. In line with Kinsbourne's predictions, spatial neglect was restored and behaviour returned to normal.

These studies demonstrate that TMS can be used to mimic a neuropsychological condition, like hemispatial neglect, in healthy volunteers. They also demonstrate that TMS can subsequently be used to reveal the neural mechanisms underlying the function that is disturbed by either the real or the virtual lesion. However, another important pillar of patient research is the constant strive to improve treatment, such as to reduce or reverse the behavioural deficit caused by a lesion. The question arising is whether TMS-induced virtual lesions can be used to explore and improve ways of treatment, using healthy volunteers under controlled experimental conditions, instead of patients. The current study takes a first step in this direction.

In the current study, we aimed to investigate in healthy volunteers whether TMS-induced extinction could be influenced by external manipulation of spatial attention during stimulus perception. In daily life we are rarely presented with an isolated stimulus in only one hemifield, rather we observe scenes in which several possibly interesting and important stimuli are simultaneously present at different locations in both visual fields, attracting and competing for our attention. Consequently, the ability to deal with competing stimuli by directing or dividing attention, and ignoring distractors if necessary, seems crucial for everyday functioning. In this light, extinction can be considered a realistic and clinically relevant experimental model for behavioural disorders of spatial attention. Since extinction occurs only when two bilateral stimuli are competing for attentional resources, directing spatial attention towards the ipsilesional hemifield prior to induction of the virtual lesion could be hypothesised to increase the strength of

induced extinction. More importantly, the other way round, if spatial attention would be directed towards the hemifield in which extinction is experienced, this could counteract the TMS-induced symptoms of extinction.

We used exogenous, peripheral attentional cues, a manipulation which has previously been shown to covertly direct spatial attention towards the left or right hemifield (Carrasco, Ling, & Read, 2004; Fu, Fan, Chen, & Zhuo, 2001). Subsequently, we induced a virtual lesion by disrupting neuronal processing in the left or right intraparietal cortex using TMS. We recorded the accuracy with which healthy volunteers reported small bilateral stimuli presented in the visual periphery. Moreover, we employed the net direction in which the percept of inaccurately reported bilateral stimuli shifted as a measure of a change in the attentional gradient. We observed an interaction, and a hemispheric difference thereof, between the location of the attentional cue, in the left or right hemifield, and the effect of TMS over left or right intraparietal sulcus on visual extinction. Our results indicate that an attentional cue presented in the contralesional hemifield can significantly reduce and thus counteract the extinction syndrome following TMS-induced parietal brain lesions. This "therapeutic" effect of directing spatial attention to the affected hemifield was of similar benefit for left and right extinction symptoms following right and left TMS-induced parietal lesions. However, an attentional cue presented in the ipsilesional hemifield significantly worsened the behavioural deficits of the virtual lesion, but only in case of a TMS-induced right parietal disruption. Thus it seems that right parietal disruption, and subsequent left hemifield extinction, is more vulnerable to an additionally presented distractor stimulus in the ipsilesional, i.e. right, visual hemifield during bilateral stimulus processing. This might not only contribute a new explanation to the reported higher prevalence of clinical symptoms following right hemispheric lesions, but it may also serve as a basis for an interesting therapeutical perspective for treatment of real lesion patients suffering from extinction. Furthermore, our study implies that healthy volunteers might not only be employable for mimicking the effects of actual brain damage, but also for evaluating the effectiveness of treatments in an early stage of development under controlled experimental conditions, thereby not immediately placing a burden on the limited amount of real lesion patients.

Methods

Participants

A total of 30 right-handed healthy volunteers with normal or corrected to normal vision (mean age: 24.2 ± 7.4), of which 9 were male, participated in this study. All participants were unaware of the goal of the study, and were naive about whether they received real or sham TMS until after having completed their participation. Before the start of each experimental session, each participant provided written informed consent and was screened for TMS experimentation safety by an independent medical supervisor. Ethical approval was given by the local medical ethical committee.

Experimental design

Participants were randomly assigned to a left hemisphere stimulation (L-IPS), right hemisphere stimulation (R-IPS), or sham stimulation group, in order to avoid carry-over and order effects. The experiment consisted of a single session, divided into a stimulus-tailoring procedure and an actual experimental part.

The tailoring procedure was similar to that employed by Hilgetag and colleagues (2001). During this procedure, participants were positioned in a head- and chin-rest and asked to fixate on the centre of the screen. During each trial of

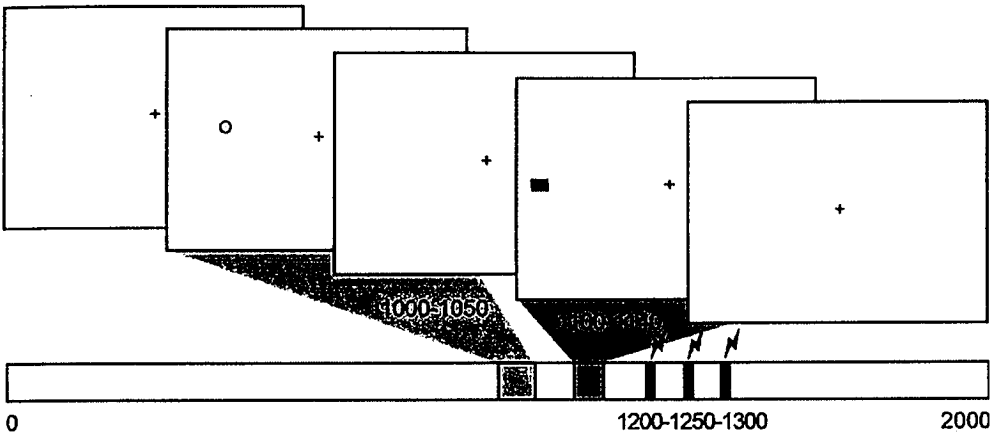

Figure 1. Experimental paradigm

After 1000 ms of fixation cross, in 2/3 of the trials an exogenous attentional cue appeared for 50 ms, at 16° eccentricity left or right or fixation. After a gap of 50 ms the target stimulus appeared for 40 ms at 24°, either unilaterally left, unilaterally right, or bilaterally. The size of the target stimuli was individually tailored according to stimulus detection threshold. In 50% of the trials three TMS pulses were delivered over left or right parietal cortex at 100, 150 and 200 ms. Participants were asked to indicate by button presses if and where they observed a target stimulus: left, right, bilaterally or no stimulus observed (no response).

the visuospatial detection task, small squares appeared shortly in the visual periphery, on the left or right side of the screen, or bilaterally. Participants were asked to indicate via button presses whether they observed a stimulus on the left, right, or bilaterally. If they did not observe any stimulus they were asked to refrain from responding. Five different stimulus sizes were presented nine times on the left, right or bilaterally, in random order. Afterwards the detection rates of each stimulus size in each condition were plotted, and the obtained response pattern of the participant was inspected to evaluate which stimulus sizes were situated around the individual detection threshold, and would thus be most suited to avoid floor and ceiling effects. The two stimulus sizes which were centred around 50% detection were selected for use in the actual experiment.

The actual experiment consisted of a similar visuospatial detection task (Figure 1), adapted from the design employed by Hilgetag and colleagues (2001). Again, participants were asked to fixate in the centre of the screen and to indicate whether and where they observed a stimulus: left, right, bilaterally or no stimulus. In addition, in 2/3 of the trials the stimulus was now preceded by an exogenous attention cue consisting of a small circle, presented slightly more central and higher compared to the square target stimulus, either left or right of fixation. 1000 ms after the fixation cross appeared, the attention cue was presented for 50 ms. Following a gap of 50 ms, the target stimulus appeared left, right or bilaterally for 40 ms. Cue and gap duration were adapted from Fu and colleagues (2001) and in line with Carrasco and colleagues (2004) among others, whereas stimulus duration was similar as in the design employed by Hilgetag and colleagues (2001). Trial onset asynchrony was jittered between 6 and 8 seconds. In 50% of the trials, 20 Hz triple-pulse TMS was applied to the left (P3, L-IPS group) or right (P4, R-IPS group) posterior parietal cortex.

Stimulation sites were determined according to the international 10-20 EEG positioning system, and have previously been shown to overlie the intraparietal sulcus (IPS) (Hilgetag et al., 2001). Existing MRI data available from a subgroup of the participants allowed us to validate with TMS neuronavigation that our P3 and P4 stimulation sites were indeed overlying the respective intraparietal sulci (Figure 2).

TMS trials were randomly intermixed with baseline trials. During a TMS trial, three TMS pulses were administered with an intensity of 150% of the individual resting motor threshold (rMT), which was determined at the start of the session by visually inspecting which TMS intensity represented the threshold for inducing movement in the index finger or thumb. rMT values measured on average 33.7 ± 6.3 % maximal machine output (MO), resulting in stimulation intensities of on average 50.5 ± 9.4 % MO. None of the participants reported any adverse

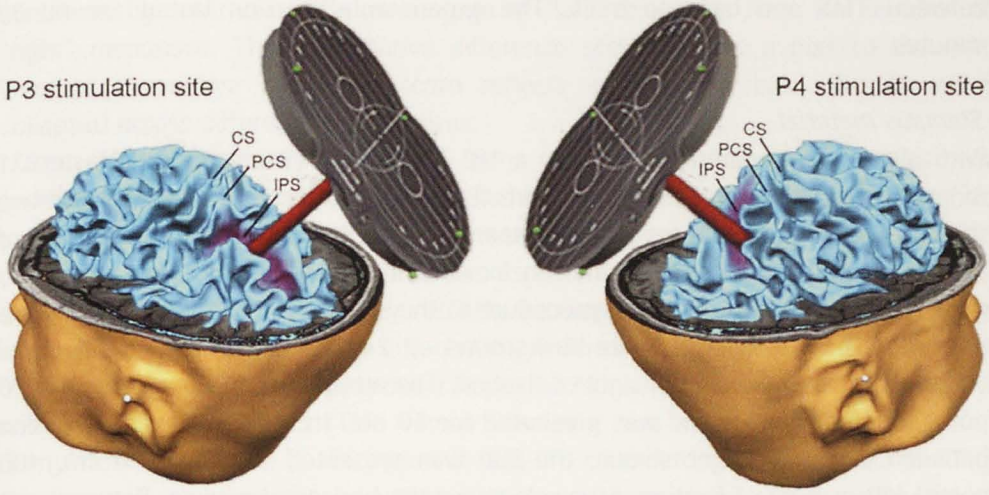

Figure 2. TMS stimulation sites
Reconstruction of the cortical surface of a single participant based on anatomical MRI data, and the TMS stimulation sites. Important sulci are indicated; CS = central sulcus, PCS = postcentral sulcus, IPS = intraparietal sulcus. The red beam represents the projection of the peak of the magnetic field. Participants assigned to the L-IPS group received TMS over 10-20 electrode position P3 (upper panel), participants assigned to the R-IPS group were stimulated over position P4 (lower panel). P3 and P4 are generally assumed to overlie left and right intraparietal sulcus, respectively. Existing MRI data available from a subgroup of the participants allowed us to validate with neuronavigation that our P3 and P4 stimulation sites were indeed overlying the respective intraparietal sulci, shown in pink.

effects. TMS pulses were delivered at 100, 150 and 200 ms after the onset of the target stimulus, a time window previously demonstrated to be maximally effective in disturbing posterior parietal cortex (Dambeck et al., 2006; Fierro et al., 2001). The TMS coil was mounted in an adjustable arm, perpendicular to the skull with the handle pointing 90° in the lateral direction. Participants wore protective earplugs throughout the experimental session.

Participants in the sham stimulation group carried out the same experiment, without attentional cueing, to control for nonspecific effects of TMS stimulation, such as a shift in spatial attention caused by the lateralised sound of TMS. Sham pulses were delivered through a magnetically shielded figure-eight coil at the fixed intensity of 50% MO, the sound level of which (67 dB) is equal to 85% MO stimulation delivered through the non-shielded experimental TMS coil. Sham stimulation was applied over P4 (hence over the same location as the R-IPS group).

The experimental session consisted of seven blocks with 36 trials each, adding up to a total of 14 trials per condition. Each block contained one catch trial in which no visual stimulus was presented. Trial onset asynchrony was jittered between 6 and 8 seconds, to avoid a slow repetitive TMS pattern and carry-over

between TMS and baseline trials. The experimental session lasted around 30 minutes.

Stimulus material

Participants were seated 40 cm from a 19" TFT screen (Samsung SyncMaster931 BF) with their head in a chin rest. Stimuli consisted of one (left or right) or two (bilateral) 60% grey squares sized between 2*2 and 4*5 pixels, with a pixel size of 0.07 mm^2. Stimulus sizes were chosen for each individual participant according to data obtained from the tailoring procedure at the start of the session. Stimuli were presented for 40 ms on a white background, at 24° (17.8 cm, 600 pixels) visual angle eccentricity left and/or right of fixation. The attentional cue consisted of a 20 point capital letter 'O' and was presented for 50 ms. In order to avoid confusion between cue and target stimuli, the cue was presented at 16° (11.8 cm, 400 pixels) left or right of fixation, 50 pixels above the horizontal midline. Between cue offset and target onset there was a temporal gap of 50 ms during which the fixation cross remained visible.

Apparatus and data acquisition

Biphasic magnetic stimulation was generated using a Medtronic MagPro 2 Tesla X100 stimulator (Medtronic Functional Diagnostics A/S, Skovlunde, Denmark). Magnetic pulses were delivered with a figure-eight-coil (Magnetic Coil Transducer MC-B70, Medtronic, wing diameter 70 mm), mounted in an adjustable arm. Sham stimulation was delivered via an otherwise identical MC-B70 coil, which has been insulated to prevent the magnetic field from passing through the scalp and entering the brain. BrainVoyager TMS Neuronavigator (Brain Innovation BV, Maastricht, the Netherlands) was used to position the TMS coil, and maintain its position throughout the experiment. Although the stimulation sites were determined based on 10-20 system coordinates, the neuronavigation system aided in precise placement and maintenance of coil position, and allowed for coil position adjustment during breaks, when necessary.

Throughout this study Presentation software (Neurobehavioral Systems, Inc., Albany, USA) was used for both stimulus presentation and recording of the behavioural responses.

Data analysis

Analyses focussed specifically on those conditions indicative for visual extinction: trials in which a bilateral stimulus was presented, but in which participants reported to have sighted only one unilateral left or right stimulus. As a dependent variable, individual difference scores were computed for each participant in each

condition, by subtracting the percentage of "left" responses from the number of "right" responses. Thus a negative difference score reflects a bias to the left, whereas a positive difference score reflects a rightward bias. Results were collapsed across different stimulus sizes.

To first establish whether there was an attentional response bias (pseudoneglect) in the absence of TMS, the aforementioned individual difference scores were entered into a one-sample t-test (test value: 0). To rule out that the three participant groups differed in this aspect, their individual difference scores were compared using a one-way ANOVA. Subsequently, we verified whether any observed attentional bias was differentially influenced by TMS over left or right intraparietal cortex, by carrying out a Repeated Measures ANOVA procedure with a within-subject factor TMS (TMS versus Baseline), and a between-subjects factor Stimulated Hemisphere (L-IPS versus R-IPS). To control for lateralised TMS shifting the locus of attention as a result of multisensory attentional cueing, a Repeated Measures ANOVA procedure was employed to compare the effects of sham TMS over right intraparietal cortex to baseline performance in the Sham TMS group. Finally, to asses the influence of exogenous attentional cues on any observed TMS-induced attentional biases, a Repeated Measures ANOVA procedure with the within-subject factors Cue (No Cue, Cue Left, or Cue Right) and TMS (TMS versus Baseline) was carried out, with Stimulated Hemisphere (L-IPS versus R-IPS) as a between-subjects factor.

Results

A one-sample t-test over individual difference scores resulting from subtracting the percentage of "left" responses from the number of "right" responses revealed a significant leftward attentional bias in the baseline condition ($t(29) = -2.807$, $p = 0.009$, 2-tailed). Hence, without TMS and without any attentional cues, an attentional bias towards the left hemifield is observed across all three groups in the baseline condition (Figure 3). This leftward attentional bias, also known as *pseudoneglect*, is frequently observed in healthy adults (Orr et al., 2005), yet systematic empirical data on this phenomenon is scarce. The leftward bias was observed similarly across the three groups, with no significant difference between them ($F(2, 27) = 0.70$, $p = 0.51$), ruling out existing differences between the two groups as an alternative explanation for any group effect described.

A subsequent Repeated Measures ANOVA revealed a significant interaction effect between TMS and stimulated hemisphere ($F(1, 18) = 4.88$, $p = 0.040$). Hence, TMS over L-IPS or R-IPS differentially influences the leftward bias observed

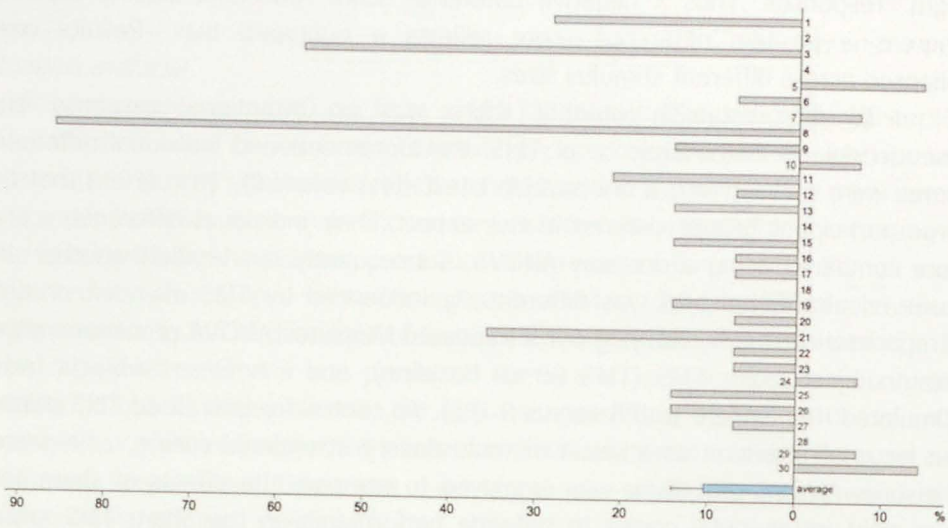

Figure 3. Pseudoneglect: leftward attentional bias in healthy adults
In healthy adults, an attentional bias towards the left hemifield has previously been described as 'pseudoneglect'. Individual difference scores were computed for each participant by subtracting the percentage of "left" responses from the number of "right" responses. A leftward extending bar (negative difference score) reflects a bias to the left, whereas a rightward extending bar (positive difference score) reflects a rightward bias. The direction and amount of attentional bias of each of the 30 neurologically healthy participants (y-axis) is displayed from top to bottom in random order, the average and its standard error of mean are depicted at the bottom in blue. On average, there is a significant leftward attentional bias in our random sample ($p = 0.007$, 2-tailed).

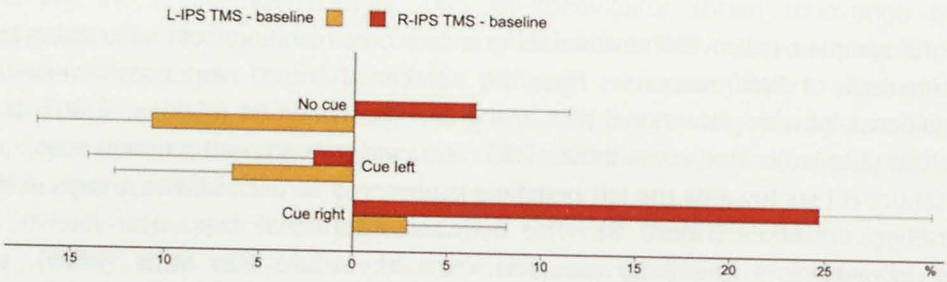

Figure 4. TMS-induced attention shifts
Effects of TMS over L-IPS (orange) or R-IPS (red), relative to baseline (no TMS). Bars represent mean individual difference scores computed by subtracting the percentage of "left" responses from the number of "right" responses. The end of each bar represents the "centre of attention" in each respective condition as a result of TMS. Zero point represents baseline (no TMS). Upper panel: left versus right parietal TMS without attentional cue, middle panel: left versus right parietal TMS with attentional cue in the left hemifield, lower panel: left versus right parietal TMS with attentional cue in the right hemifield.

in the baseline condition. L-IPS TMS, without attentional cues, induces visual extinction of stimuli in the right hemifield (Figure 4) and thus induces an even more leftward bias than in the baseline condition (increased pseudoneglect), whereas R-IPS TMS reverses the pseudoneglect by inducing visual extinction of stimuli in the left hemifield and significantly shifting attention more rightward compared to baseline (Figure 4). Sham TMS over the same right intraparietal stimulation site did not result in any shift of attention ($F(1, 9) = 0.011$, $p = 0.92$), ruling out nonspecific effects of TMS as an alternative explanation. These findings replicate earlier findings describing extinction induced by repetitive TMS over the same stimulation sites (Dambeck et al., 2006; Hilgetag et al., 2001).

Finally, a Repeated Measures ANOVA including the effect of attentional cueing on the previously described TMS-effects revealed a significant interaction between Stimulated Hemisphere and TMS ($F(1, 18) = 4.52$, $p = 0.048$), as well as between Cue and TMS ($F(2, 17) = 4.75$, $p = 0.023$). A visualisation of the observed results is depicted in figure 4. After a leftward attentional cue, both L-IPS and R-IPS TMS groups show an attentional shift towards the left. After a rightward cue, L-IPS induces an attentional shift to the contralesional right hemifield, whereas R-IPS induces a massive rightward bias compared to baseline, even extending the centre of attention far into the right hemifield.

Discussion

Transcranial magnetic stimulation has already successfully been used to mimic the behavioural deficits following brain lesions, as well as for unravelling the mechanisms underlying some of these deficits. In the current study we aimed to explore whether TMS could also be employable to probe the effectiveness of treatments in an early stage of development, in healthy adults and under controlled experimental conditions, thereby sparing the limited amount of real acute lesion patients. More specifically, we investigated whether externally manipulating covert spatial attention influences TMS-induced virtual extinction in healthy volunteers.

In response to bilateral stimuli presented under neutral baseline conditions, we observed a clear leftward attentional bias across all three of our groups of healthy participants (Figure 3). This uneven distribution of covert spatial attention has been described before and is commonly known as pseudoneglect, or in this case pseudo-extinction. The shift of the centre of attention well into the left visual hemifield could be explained, according to Corbetta (2002), as a result of a right hemispheric dominance with regard to spatial attention, which pulls spatial

attention towards the left in neutral baseline conditions. However, in line with Kinsbourne's (1993) theory, it could also be explained as the result of a shifted attentional gradient resulting from interhemispheric inhibition, whereby the right hemisphere exerts stronger inhibition onto the left hemisphere, than vice versa.

It is by now well-established that application of TMS over parietal cortex has the ability to temporarily induce visuospatial deficits like neglect and extinction. In many previous studies visuospatial deficits were, in line with neuropsychological patient data, established more strongly after right parietal TMS, while no such effects are observed following left parietal TMS. In some of these studies, left parietal TMS exerted no behavioural effect on stimuli presented in the right visual field (see e.g. Chambers, Payne, Stokes, & Mattingley, 2004; Dambeck et al., 2006; Fierro et al., 2000; Fierro et al., 2001; Hilgetag et al., 2001). In the current study, we found extinction of the contralesional part of a bilateral stimulus after right as well as after left parietal TMS. It is still highly debated whether extinction is merely a symptom of hemineglect, or whether it constitutes a different visuospatial defect altogether (Pavlovskaya et al., 2007; Smania et al., 1998). One aspect in which neglect and extinction seem to differ is the degree of lateralisation of the symptoms; whereas neglect is well-known to affect almost exclusively the left visual hemifield, extinction seems to be less confined to one hemifield. Our observation that both right and left parietal TMS induce extinction of the contralesional part of a bilateral stimulus further contributes to this ongoing discussion about the proposed lateralisation of extinction, by showing that this "virtual extinction" seems to occur equally strong after left and right parietal TMS.

Knowing that TMS is able to induce temporary behavioural deficits, so-called virtual lesions, we wanted to investigate whether TMS could possibly also enlarge our insights into the workings and applicability of rehabilitive treatment approaches. In a subsequent stage of data analysis we therefore looked at interactions between the aforementioned effects of right versus left parietal TMS, and the external manipulation of covert spatial attention using a brief visual cue, presented in either the left or right visual hemifield. While a visual cue presented in the left hemifield shortly before target stimulus onset did not further increase the leftward bias caused by left parietal TMS, a visual cue presented in the right hemifield showed a significant interaction with the rightward bias caused by right parietal TMS. More specifically, a rightward visual cue substantially increased the extinction of left stimuli induced by right parietal TMS, to a degree in which on average 25% more bilateral stimuli were misperceived as unilateral right stimuli, compared to neutral baseline, and about 22% percent more compared to right parietal TMS alone. This implies that directing covert attention towards the right hemifield can aggravate left hemifield extinction induced by right parietal TMS. The

fact that we did not find a similar aggravation of right hemifield extinction induced by left parietal TMS after directing covert attention towards the left hemifield coincides with the extensive body of neuropsychological literature showing a strong right hemispherical lateralisation of hemispatial neglect and extinction. Although both left and right parietal TMS result in contralateral extinction, implying equal vulnerability in both hemispheres, only left hemifield extinction following right parietal TMS is severely aggravated by a competing stimulus in the ipsilesional (right) hemifield. Apparently, a competing stimulus in the right hemifield captures attention in such a fashion that it severely deteriorates the ability to subsequently redistribute attention also towards the contralesional left hemifield when a bilateral stimulus is presented. This could signal an inability to detach attention from the ipsilesional hemifield once it has been drawn there by a visual stimulus, be it a visual cue, or an object that catches attention in a natural scene. These observations speak to a model proposing an incapacity with regard to the redistribution of attention in the presence of an attention-binding distractor as a possible catalyst underlying the deficits characteristic of extinction, and maybe also one of the neural mechanism accounting to the hemispheric asymmetry of attention deficits following unilateral brain lesions. Furthermore, it seems that only after disruption of right parietal -as opposed to left parietal- cortical functioning a distractor presented in the ipsilesional hemifield poses an insurmountable problem for detecting the contralesional counterpart of a subsequent bilateral stimulus. We put forward the possibility that the asymmetry with respect to this ability of detaching attention from a distractor, an ability crucial in almost every daily life setting, is contributing to the well-described right hemispheric lateralisation with regard to extinction. The mixed empirical reports with regard to the lateralisation of extinction could be related to a less or more severe disruption of the attention redistributing ability. In line with the fact that double dissociations between hemispatial neglect and extinction are observed frequently, our results further support the notion that these symptoms are not part of the same condition.

Hence, the observation that specifically left hemifield extinction induced by right parietal TMS can be aggravated by cueing covert spatial attention towards the right hemifield reveals new and important information about the cortical processes underlying bilateral extinction. However, from a therapeutic perspective, it is even more interesting to explore whether directing covert spatial attention towards the contralesional hemifield could also counteract TMS-induced extinction rather than aggravating it. Indeed, a visual cue presented in the left hemifield effectively drew the centre of attention away from the right hemifield, thus counteracting the extinction of left hemifield stimuli caused by right parietal TMS. Similarly, a visual cue presented in the right hemifield counteracted the right

hemifield extinction brought about by left parietal TMS. Thus it seems that visual cues in both contralesional hemifields can counteract the behavioural impairments brought about by a TMS-induced virtual lesion to the respective contralateral parietal cortices. Consequently, both potentially have therapeutic value. However, since in practice behavioural impairments are often restricted to the left hemifield, presenting cues in the left visual field potentially provides the most therapeutic gain. Future studies should aim at validating and further exploring the possible therapeutic benefits of attentional cueing in treating extinction patients, also extending to the effects of central visual cues or crossmodal cues, which might be even more efficient than unisensory visual cues (Spence, 2010).

In conclusion, our findings imply that externally manipulating covert spatial attention can enhance or counteract symptoms of extinction induced temporarily by parietal TMS. The enhanced symptoms of left hemifield extinction after right parietal TMS after a right attention cue specifically, might reflect a disruption of the ability to detach attention from a distracting cue and redistribute it over both hemifields in order to correctly perceive a bilateral visual stimulus. This ability is very likely right lateralised, thus contributing to the right hemispheric lateralisation of extinction. In addition, a visual cue presented in the contralesional hemifield shortly before a bilateral visual stimulus appears can reduce TMS-induced extinction of the contralesional counterpart of the bilateral stimulus. Whether this is also effective in patients suffering from chronic behavioural impairments caused by actual brain lesions remains to be studied, but if so the manipulation of spatial attention could possibly prove useful for therapeutic interventions in the future. On a broader level, our results show that TMS might not only be used to evaluate which behavioural impairments occur after disruption of a certain brain area, the so-called virtual lesion approach, and which cortical processes are underlying the disturbed functions, but that TMS might also be employable as a tool for probing and evaluating rehabilitive treatment options in an early stage. Such an approach increases explorative possibilities, limits the burden placed on the small amount of available acute lesion patients, and drastically reduces the impact of the many confounding factors inevitably associated with studying patients.

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Chapter 4

The sound of size

Crossmodal binding in pitch-size synesthesia: a combined TMS, EEG and psychophysics study

Based on:
Bien, N.[#], Oever, S. ten[#], Goebel, R., and Sack, A.T. (submitted). The sound of size. Crossmodal binding in pitch-size synesthesia: a combined TMS, EEG and psychophysics study. [#] Authors contributed equally to the study

Abstract

Crossmodal binding usually relies on bottom-up stimulus-characteristics such as spatial and temporal correspondence. However, in case of ambiguity the brain has to decide whether to combine or segregate sensory inputs. We hypothesise that widespread, subtle forms of synesthesia provide crossmodal mapping patterns which underly and influence multisensory perception. Our aim was to investigate if such a mechanism plays a role in the case of pitch-size stimulus combinations. Using a combination of psychophysics and ERPs, we could show that despite violations of spatial correspondence, the brain specifically integrates certain stimulus combinations which are congruent with respect to our hypothesis of pitch-size synesthesia, thereby impairing performance on an auditory spatial localisation task (Ventriloquist effect). Subsequently, we perturbed this process by functionally disrupting a brain area known for its role in multisensory processes, the right intraparietal sulcus, and observed how the Ventriloquist effect was abolished, thereby increasing behavioural performance. Correlating behavioural, TMS and ERP results, we could retrace the origin of the synesthestic pitch-size mappings to a right intraparietal involvement around 250 ms. The results of this combined psychophysics, TMS and ERP study provide evidence for shifting the current viewpoint on synesthesia more towards synesthesia being at the extremity of a spectrum of normal, adaptive perceptual processes, entailing close interplay between the different sensory systems. Our results support this spectrum view of synesthesia by demonstrating that its neural basis crucially depends on normal multisensory processes.

Introduction

In daily life, perception of objects and scenes is rarely unisensory in nature. In fact, any given percept often stimulates multiple senses at the same time, most commonly the visual and auditory modalities. In a world which is highly complex in both the visual and auditory domain, accurately combining or segregating these simultaneous sensory inputs from different modalities is one of the crucial processes in making sense of the world surrounding us. The key function of multisensory integration is to dissociate between stimuli originating from different sources, which should be treated as separate perceptual objects, and stimuli originating from a single source, which should subsequently be perceived and treated as a single object of perception. This raises the question when and how multimodal inputs are combined into a single percept. In contrast to unisensory neurons found in sensory cortices, there are also brain regions containing multisensory neurons, which have spatially overlapping receptive fields in two or more modalities. Different sensory inputs are integrated if they simultaneously activate both receptive fields of a multisensory neuron, thus if they are temporally and spatially congruent (Wallace et al. 1996). Neurons with such multisensory properties have been localised in deep layers of the primate superior colliculus (Wallace, Wilkinson et al. 1996). In humans, cortical areas displaying similar characteristics were found in the superior temporal cortex (van Atteveldt et al. 2004) and in the parietal cortex (e.g. Calvert 2001; Calvert et al. 2000; Calvert et al. 2001).

In case of sensory inputs which are ambiguous with regard to one of the criteria of spatial and temporal correspondence, the brain has to decide whether or not to integrate these inputs into a single percept. Under these circumstances, top-down cognitive mechanisms may overrule bottom-up differences between temporal onset and/or spatial origin of the different sensory inputs. Of course some top-down couplings could be attributed to repeated exposure to different sensory counterparts of an event, being perceived together constantly over and over again, until an overlearned semantic link is formed. This might be how we learn that a dog barks, whereas a cat miaows – and not the other way around. This overlearned pairing could possibly exert top-down influence on the binding process in case of bottom-up ambiguity. However, repeatedly perceiving some stimuli in accordance with each other cannot explain why different days have different colours to different people (Simner et al. 2006), why white balls squeak (Mondloch and Maurer 2004), why small is bright and big is dark (Cohen Kadosh et al. 2007), why small numbers are positioned in the left half of mental space (Dehaene et al. 1993) – in short, why some stimuli, which are at first glance not obviously related,

are consistently linked by multisensory mechanisms which are thought to be operating from a bottom-up perspective. Even to a level at which multisensory integration actually deteriorates performance (e.g. Driver 1996; McGurk and MacDonald 1976; Parise and Spence 2009).

Apparently, certain automatic crossmodal mapping patterns occur in the normal population, yet bear semblence of the rare phenomenon of synesthesia. While synesthesia literally means "joining of the senses", it is commonly referred to as a sensory perception in a modality additional to the one which was physically stimulated (see e.g. Hubbard et al. 2005). Although it has always fascinated the general public, scientifically synesthesia represents a rather underexposed perceptual phenomenon. There is growing consensus that the research of synesthesia could advance our understanding of the normal and abnormal human brain and cognition (e.g. Cohen Kadosh and Henik 2007; Mulvenna and Walsh 2006). Extreme, rather rare forms of synesthesia like grapheme-colour synesthesia have traditionally received most scientific attention. This leads to a view of synesthetic perception, and its underlying mechanisms, as being qualitatively different from normal multisensory integration. However, examples of systematic crossmodal mapping which cannot be explained simply by bottom-up stimulus characteristics could be interpreted as milder forms of synesthesia (Martino and Marks 2001), which are not just rare anomalies, but rather adaptive and widespread traits in the normal population. One example is that of the "squeaking white balls", described by Mondloch and Maurer (2004). They observed young children consistently appointing a small sized or light coloured ball as the source of a high pitched tone, and vice versa. There is increasing evidence that many of such common mapping patterns between e.g. pitch, size, colour and distance (Cohen Kadosh et al. 2008; Parise and Spence 2008; Romei et al. 2009) are retained into adulthood. For example, one widespread form of synesthesia is the mental number line, a spatial distribution of small and large numerical values across horizontal mental space (Dehaene, Bossini et al. 1993) which can modulate spatial attention (Fischer et al. 2003).

Besides such crossmodal mappings, which seem to rely on certain top-down binding characteristics, reports of synesthesia and crossmodal integration sharing common neural mechanisms also hint at a non-qualitative distinction. For example, TMS disruption of parietal cortex disrupts automatic integration of synesthetic mappings between graphemes and colours in grapheme-colour synesthetes (Esterman et al. 2006; Muggleton et al. 2007). Parietal cortex has also been reported in other studies into synesthesia (Beeli et al. 2008; Rouw and Scholte 2010; Weiss et al. 2005). Interestingly, the same brain region is known for

its role in multisensory integration (Calvert, Hansen et al. 2001; Werner and Noppeney 2010).

Moreover, whereas synesthetic mappings were initially considered random, coincidental associations, it turns out that there are recurrent patterns across individuals with regard to, for example, grapheme-colour associations (Brang et al. 2011; Eagleman 2010; Rich et al. 2005), and across languages and alphabets (Brang, Rouw et al. 2011; Cohen Kadosh, Henik et al. 2007; Eagleman 2010; Kim 2010). Similar synesthetic mapping patterns have also been reported across synesthetes and non-synesthetes (Spector and Maurer 2008; Ward et al. 2006). Apparently, there are shared underlying representations for at least certain synesthetic patterns. The purpose of such shared mapping patterns has yet to be explored, however it is likely that they are to some extent meaningful and advantageous.

In addition, there is the well-described difference between so-called 'associator' versus 'projector' synesthetes with regard to their qualitative experiences, and the way synesthesia influences their performance on psychophysical tasks (Dixon et al. 2004; Hubbard and Ramachandran 2005; Muggleton, Tsakanikos et al. 2007; Rouw and Scholte 2010). Projectors' sensory byproducts interfere stronger with performance on a synesthetic Stroop task than the associations reported by associator synesthetes (Dixon, Smilek et al. 2004). It appears that synesthesia is not an all-or-nothing condition, as it has traditionally been considered, but contrarily there is variability with regard to how much synesthesia a synesthete experiences, and consequently, to what degree their synesthesia modulates automatic perceptual processes.

As a result, viewpoints have recently been shifting more towards synesthesia being at the extremity of a spectrum of normal, adaptive perceptual processes, entailing close interplay between the different sensory systems (Eagleman 2009; Esterman, Verstynen et al. 2006; Martino and Marks 2001; Mulvenna and Walsh 2006; Nikolic 2010; Sagiv and Ward 2006). Consequently, the concept and the criterion of what is termed synesthesia might need to be expanded (Nikolic 2010). In line with this emerging spectrum view of synesthesia, we propose that synesthesia and crossmodal integration are indeed closely linked. In fact, we suggest that when simple criteria such as temporal and spatial congruency fail to explain why sensory stimuli are integrated or not, synesthetic processes become of relevance by providing intrinsic mappings which allow for top-down influence on the integration process. Synesthesia as such can be seen as a crossmodal process which relies on neural structures shared with normal crossmodal processing, which can manifest itself in different strengths, exerting graded influence on perception as well as on modulation of automatic processes,

which entails shared and possibly meaningful mapping patterns across individuals, and which has many milder, widespread manifestations in the normal population. The spectrum view of synesthesia meets all these criteria, with common, prevalent instances of crossmodal mapping as the 'missing link' between normal multisensory integration and extreme forms of synesthesia. Although even extreme forms of synesthesia have been found to adhere to certain rules or regularities, it is most likely the variety of common, widespread forms which can inform us best on yet unacknowledged crossmodal processes which are clearly underlying and influencing everyday perception.

In the current study we tried to deepen our understanding of common synesthetic mappings in the normal population. To this end, we employed the Ventriloquist paradigm (Driver 1996): when presented with spatially segregated but temporally and semantically congruent audiovisual speech, the brain decides that because sound and vision are simultaneous and congruent, they must belong to the same source. Since human visual perception is more accurate in the spatial domain than auditory perception, the spatial origin of the speech sound is misallocated to the location of the lip movements, leading the brain to believe that visual and auditory speech originated from the same spatial source. A similar ambiguous situation arises when sensory stimuli belonging to multiple sensory events arrive simultaneously from approximately the same location. Again, the brain has to decide whether or not to integrate some of these stimuli, and which ones belong together. The Ventriloquist illusion, in line with other ambiguous multisensory settings in which the brain decides to integrate sensory events despite violation of the basic rules of correspondence (e.g. McGurk and MacDonald 1976), or vice versa, raises the question which type of information is of crucial importance. Clearly, basic bottom-up stimulus characteristics such as temporal and spatial correspondence are not always sufficient to explain the 'when' and 'when not' of multisensory integration.

This paradigm allowed us to systematically probe the multisensory system by deliberately violating basic bottom-up rules of integration and enabled us to systematically and quantitatively verify whether small sized objects and high pitches are grouped together (henceforth labelled "synesthetically congruent"), as compared to small objects and low pitches, in randomly selected volunteers. If a small object and a high pitch are indeed considered highly semantically congruent, the brain would group them together notwithstanding the spatial separation between them, which would result in decreased accuracy in determining the spatial origin of the tone with regard to the visual stimulus. In a next step, we verified whether multisensory integration plays a crucial role in binding these percepts, by using repetitive transcranial magnetic stimulation (TMS) in order to temporarily

disrupt the right intraparietal cortex, a brain region previously reported of relevance for both synesthetic and crossmodal binding processes. During task execution we recorded brain activity with 64 EEG-electrodes to provide a detailed temporal overview of neural processes reflecting synesthetic mapping, as well as to assess the neurophysiological correlate underlying TMS-induced changes in synesthetic behaviour. This combination of psychophysics, ERP and TMS revealed significant interactions between synesthesia-mediated behaviour and ongoing neuronal processes in higher perceptual brain areas within frontal and parietal cortex.

Methods

Participants

A total of 14 neurologically healthy volunteers with normal or corrected to normal vision (aged between 18 and 25), 4 of which were male, participated in this study. Eleven of these participants simultaneously underwent EEG recording. All participants were unaware of the goal of the study until after having completed their participation. Before the start of each experimental session, each participant provided written informed consent and was screened for TMS experimentation safety by an independent medical supervisor. Ethical approval was given by the local medical ethical committee. Participants were rewarded with student participation credits.

Paradigm and psychophysics procedure

Each experimental participation consisted of two sessions, separated by at least one day. The first session consisted of two parts: a tailoring procedure was carried out before the actual experimental session commenced.

The tailoring procedure at the start of the first session was carried out to ensure that the difficulty level of the experimental task was similar for each participant, regardless of inter-individual differences. Participants were asked to fixate at the centre of a screen, and indicate via a button press whether they thought a tone originated from a location left or right of fixation. Their individual auditory localising threshold was determined using a staircase procedure. During this tailoring procedure, participants were comfortably seated with their head in a chin rest, at 55 cm viewing distance from the computer screen.

After the tailoring procedure, EEG preparation and (depending on the session) TMS application were finalised, the actual experimental session started. Again, participants were asked to fixate at the centre of the screen. In each trial, a

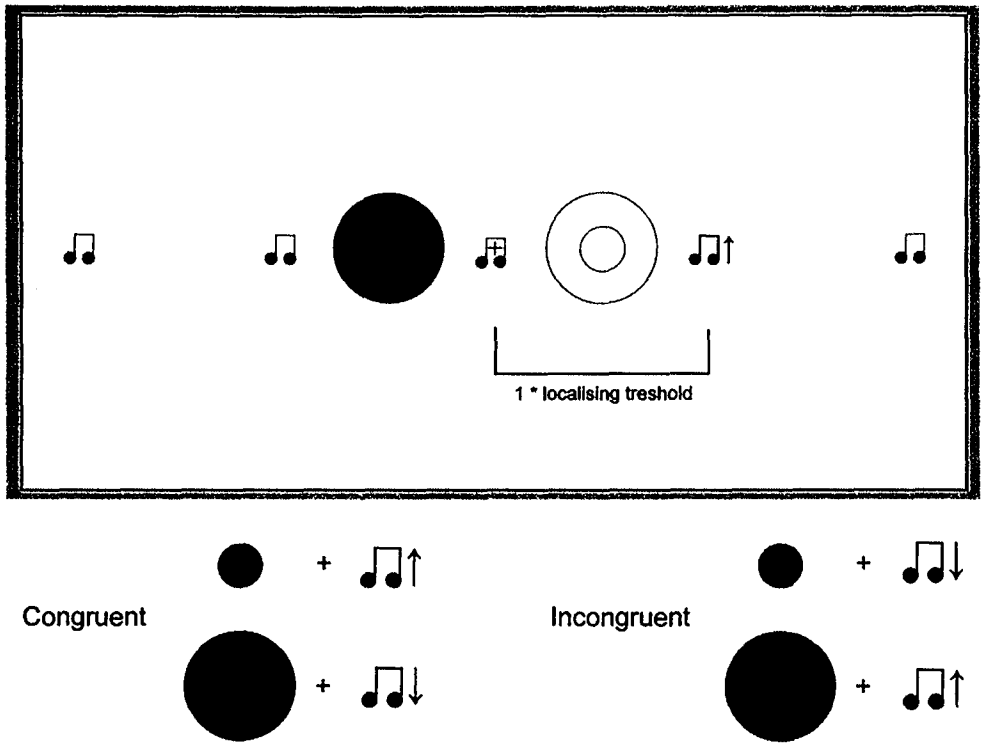

Figure 1. Pitch-size ventriloquist paradigm
Simultaneously with the visual presentation of a circle with a diameter of either 5.2° (large) or 2.1° (small) of visual angle, a high (4500 Hz) or low (250 Hz) tone was presented at one out of five possible locations. Participants indicated whether the tone originated from the left or, as in the example shown, from the right of the visual stimulus. Visual and auditory stimulus could either be congruent (small-high or large-low) or incongruent (small-low or large-high) according to the hypothesis of pitch-size synesthesia. Stimulus locations were tailored according to individual auditory localising thresholds.

visual circle stimulus appeared left or right of fixation, while concurrently a pure tone auditory stimulus was emitted from two speakers flanking the screen. Both stimuli lasted 200 ms and differed in spatial location. The visual stimulus could be either large or small, and the tone could have a low or a high pitch (Figure 1). The combination of visual size and tone pitch could be either congruent according to the hypothesis of pitch-size synesthetic mappings in the normal population (large stimulus – low pitched tone or small stimulus – high pitched tone), or incongruent (large stimulus – high pitched tone or small stimulus – low pitched tone). Participants were asked to make a forced-choice judgment on the spatial origin of the tone, either left (right index finger response) or right (right middle finger response) of the visual stimulus. Accuracy and reaction times of the responses were recorded.

The experiment consisted of 320 trials per session divided over four blocks of approximately four minutes each. Trial onset asynchrony was randomly jittered between 3 and 4 seconds. Condition order was randomised, with the restriction that an equal amount of trials was presented in each condition throughout the whole study. Hence the number of trials in each condition was fully balanced. A 2 by 2 factorial design was employed, with the conditions Synesthetic Congruency (congruent or incongruent) and TMS (TMS or no TMS) as the two within-subject factors.

Stimulus material

Auditory stimuli consisted of high (4500 Hz at 63 dB) or low (250 Hz at 72 dB) pitched pure tones, lasting for 200 ms. Sound levels were chosen in accordance with equal loudness curves (Suzuki & Takeshima, 2004), assuring that the perceived loudness for the high and low pitched tones was equal. The sounds were presented by two loudspeakers placed on both sides of the computer screen. By systematically varying the ratio between the loudness produced by each of the two speakers, five illusory auditory locations were created on a horizontal axis (Figure 1). These locations were determined according to each participant's individual auditory localising threshold, as detected in a staircase tailoring procedure (see section design and procedure). Auditory stimuli were situated left or right of fixation at one or two times of the individual threshold distance. The fifth location was the centre of the screen itself. To increase difficulty, and thus maximise multisensory integration processes (Meredith & Stein, 1986; Stein, Stanford, Ramachandran et al., 2009), white noise with an intensity of 65 dB was presented in the background during the entire experimental session.

Visual stimuli consisted of a large (5.2° visual angle) or small (2.1° visual angle) 20% grey circle, presented also for 200 ms on a 17" TFT screen (Samsung SyncMaster 931 DF) on white background. Visual stimuli were presented at distances 0.5 times the individual auditory localising threshold left or right of fixation. Throughout the experiment a 20% grey fixation cross was shown in the centre of the screen. Presentation software (Neurobehavioural Systems, Inc., Albany, NY) was used for both stimulus presentation and recording of the behavioural responses.

TMS design and apparatus

During one of the two sessions, the order of which was randomised between participants, continuous theta burst TMS (Huang et al., 2005) was administered over the right parietal P4 EEG electrode position (10-20 EEG positioning system), which has been shown to overlie intraparietal sulcus (see e.g. Hilgetag et al.,

2001). Biphasic magnetic stimulation was generated using a Magstim Rapid^2 stimulator (The Magstim Company, Whitland, UK). Magnetic pulses were delivered with a hand-held figure of eight coil (70 mm standard coil, The Magstim Company, Whitland, UK) placed tangentially to the scalp with the handle pointing 45 degrees in the lateral-inferior direction.

Continuous theta burst TMS is an inhibitory patterned TMS protocol, applied over the course of 40 seconds, with an effectivity outlasting the stimulation itself by approximately 1 hour (Huang et al., 2005). Application of TMS is inevitably accompanied with specific side-effects. The clicking sounds as well as the sensations on the scalp can be distracting during behavioural task execution, and delivering the magnetic pulse distorts ongoing EEG recording. Employing offline theta burst TMS allows for TMS artefact-free recording of behavioural as well as EEG data. Stimulation was delivered at 80% of the individual resting motor threshold (with a maximum not exceeding 45% of the maximum output of the stimulator). Effectively, participants were stimulated on average at 70.8% of their individual resting motor threshold. To ensure that the offline effects of TMS did not affect behaviour during the no TMS condition, sessions were at least two days apart.

EEG apparatus and data acquisition

ERPs were recorded via a 64 Ag-AgCl electrodes BrainCap MR EEG cap (BrainProducts GmbH, Munich, Germany) and a BrainAmp MR Plus EEG amplifier (BrainProducts GmbH, Munich, Germany) with a sampling rate of 1000 Hz. Online EEG recording was performed with BrainVision Recorder (BrainProducts GmBh, Munich, Germany). All recordings were referenced online to the Cz electrode. Grounding was provided by a separate electrode located posterior-centrally on the head. The vertical electro-oculogram (VEOG) was recorded from electrodes placed above and below the left eye. EEG preparation was completed before TMS was applied and lasted approximately one hour. All electrodes were inserted with Abralyt 2000 electrolyte gel (Easycap, Germany) and subsequently fiddled with a small wooden stick until impedance was below 50 kΩ.

Behavioural and TMS data analysis

Outliers and trials with a reaction time below 200 ms post stimulus presentation were discarded from further analysis. Trials were labelled as synesthetically congruent or incongruent based on the hypothesis of pitch-size synesthetic mappings. In order to first verify whether pitch-size synesthesia was indeed a common trait within our sample of non-synesthetic participants, mean values of accuracy per participant, per condition, were taken from the session without TMS

and entered into a Repeated Measures ANOVA procedure with the within-subject factor Synesthetic Congruency (synesthetically congruent or incongruent). Subsequently, the effect of TMS on pitch-size synesthesia was evaluated by entering mean values of accuracy per participant, per condition into a two-way Repeated Measures ANOVA procedure with the within-subject factors Synesthetic Congruency (synesthetically congruent or incongruent) and TMS (TMS or no TMS). The same analyses with an additional factor Accuracy (correct or incorrect) was applied to the reaction time data. Alpha values of pair-wise comparisons were Bonferroni corrected to correct for multiple comparisons.

ERP data analysis

EEG data were pre-processed and analysed using Brain Vision Analyzer 2.0 (Brain products, Munich, Germany). Data was re-referred offline to the algebraic average reference and filtered with a 50-Hz notch filter and band-pass filter (0.5-70Hz, 12dB/oct). Continuous EEG data were divided into epochs ranging from -500 to 1500 ms relative to stimulus onset. Baseline was corrected using 200 ms of pre-stimulus activity as a reference. Artefacts were automatically detected and manually checked through visual inspection. Artefacts were removed per individual channel. Subsequently epochs were averaged per condition and combined into a group average for each condition, which was subsequently used for data analysis.

Automatic peak detection was employed in four ERP components of interest: P1 (90-150 ms), N1 (150-250 ms), P2 (200-300 ms) and N3 (250-350 ms). Resulting mean amplitudes within an interval of two milliseconds around each peak were used for further analysis. These amplitudes were collapsed into two spatial clusters of electrodes: frontal (electrodes Fz, F1, F2, F3, F4, AFz, AF3, AF4, AF7, AF8, FPz, FP1, FP2, FC3 and FC4) and parietal (electrodes CP1, CP3, P3, CP5, P5, CP4, CP2, CP6, P4, P6, Pz, P2 and P1). To first establish how pitch-size synesthesia is reflected in brain potentials, without the influence of TMS, the resulting mean amplitudes of the four time bins within these two clusters were subsequently used as dependent variables in a Repeated Measures Multivariate ANOVA (RM MANOVA) with the factor Congruency (congruent or incongruent). Additionally, the effect of right parietal TMS on the observed effects of synesthetic congruency was evaluated using a two-way RM MANOVA with the factors Congruency (congruent or incongruent) and TMS (TMS or no TMS). Post hoc analyses were conducted for the interaction effect of Congruency per level of TMS.

In order to get more insight into the nature of the interaction between the ERP components, we additionally calculated Pearson product-moment correlation coefficients between the relevant components parietal N1, frontal P2, parietal P2 and frontal N3.

Combined behavioural-ERP analysis

To determine which ERP component reflects pitch-size synesthesia and its modulation after TMS, we calculated Pearson product-moment correlation coefficients between the four aforementioned ERP components and behavioural accuracy over the four different conditions. Since interactions between the factors Congruency and TMS could not be incorporated in the correlation analysis directly, we computed individual incongruent minus congruent accuracy difference scores for No TMS and TMS separately, and correlated those with the equivalent ERP component amplitude difference scores. Considering that TMS is an experimental manipulation of brain function, this information provides direct information about the neural correlates of observed behavioural effects.

Results

Behavioural and TMS results

Two participants showed an opposite behavioural pattern, and were thus discarded from further analyses, with the exception of correlational analyses which rely on different statistical assumptions. In accordance with the hypothesis of pitch-size synesthesia in the normal population, synesthetically congruent trials were defined as those which combined a high pitched tone with a small visual stimulus or a low pitched tone with a large visual stimulus, and synesthetically incongruent trials as those which combined a high pitched tone with a large visual stimulus or a low pitched tone with a small visual stimulus. If pitch-size synesthesia is indeed a common trait in the normal population, multisensory integration resulting from the Ventriloquist illusion would decrease the perceived spatial distance between the synesthetically related auditory and visual source in the congruent condition, thereby hampering auditory localisation in synesthetically congruent as compared to synesthetically incongruent trials.

Without TMS, participants were indeed more accurate on synesthetically incongruent trials as compared to congruent trials ($F(1, 11) = 23.332$, $p < .01$, $\eta p^2 = .680$) (Figure 2A). As hypothesised, judging the spatial origin of the auditory relative to the visual stimulus was more difficult when stimuli were synesthetically congruent according to our hypothesis of pitch-size mappings in the normal population. Hence, automatic integration of synesthetically related audiovisual stimuli hindered an accurate spatial judgment of the auditory stimulus with regard

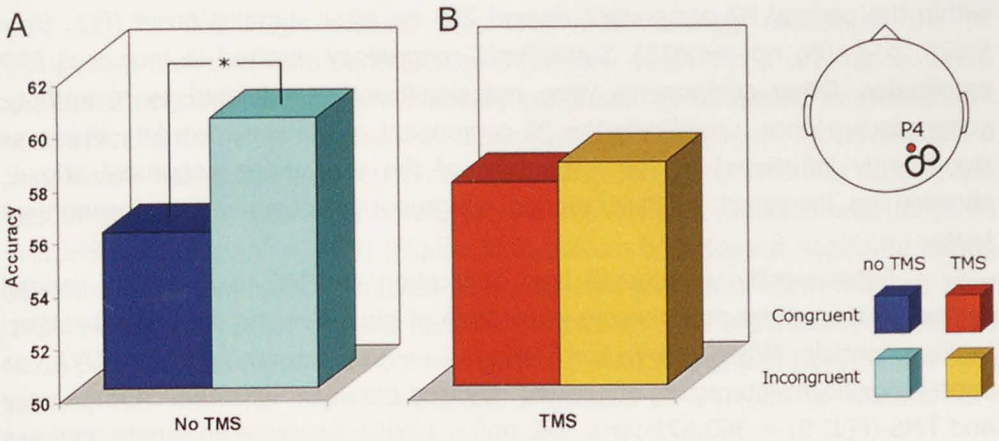

Figure 2. Behavioural results

A) Behavioural results reflecting the significant interaction effect between synesthetic congruency and TMS over right parietal cortex on accuracy in an auditory spatial localising task. Asterisks indicate significant effects ($p < 0.05$, Bonferroni corrected). Without TMS participants were less accurate in dissociating the spatial origin of a tone when visual stimulus and tone were synesthetically congruent. After TMS this difference disappeared almost completely.

to the visual stimulus. Reaction times did not show any significant effects, ruling out speed-accuracy trade-off accounts for the observed accuracy differences.

Application of TMS to the right parietal cortex significantly interacted with this Synesthetic Congruency effect ($F(1, 11) = 6.637$, $p < .05$, $\eta p^2 = .376$), and thus crucially changed the observed behavioural difference pattern. While without TMS participants were significantly less accurate in synesthetically congruent trials, this adverse effect of synesthetic congruency was significantly diminished after disruption of right parietal cortex ($F(1, 11) = .253$, $p = 1.00$, $\eta p^2 = .000$) (Figure 2B). In other words, after right parietal TMS, automatic integration of synesthetic congruent stimuli no longer hampered auditory spatial localisation judgment, supposedly because the automatic, synesthetically induced multimodal integration of these stimuli was prevented. Importantly, TMS did not affect overall accuracy ($F(1, 11) = .000$, $p = .987$, $\eta p^2 = .000$), discarding nonspecific TMS effects as an account for the observed phenomenon.

ERP results

Firstly, by evaluating simple effects of Synesthetic Congruency without TMS, we investigated how pitch-size synesthesia was embodied by event-related brain potentials. Although the overall multivariate effect of Synesthetic Congruency was not significant in a RM MANOVA without TMS ($F(1, 9) = 7.585$, $p = .274$, $\eta p^2 = .984$), univariate analyses showed a significant effect of synesthetic congruency

within the parietal P2 component around 250 ms after stimulus onset ($F(1, 9) = 5.626$, $p < .05$, $\eta p^2 = .413$). Synesthetic congruency resulted in increased ERP amplitudes. Other components were not significantly modulated by synesthetic congruency. Hence, specifically the P2 component at parietal recording sites was significantly influenced by the integration of the synesthetic congruent stimuli, showing an increased positivity during congruent as compared to incongruent trials.

Subsequently, we tested how application of TMS to the right parietal cortex influenced the multisensory integration of pitch-size stimuli in event-related brain potentials. When the factor TMS was entered into the RM MANOVA, an overall significant interaction effect was revealed between Synesthetic Congruency and TMS ($F(1, 9) = 360.671$, $p < .05$, $\eta p^2 = 1.000$). Separate univariate analyses over mean amplitudes per electrode cluster (parietal and frontal) per component

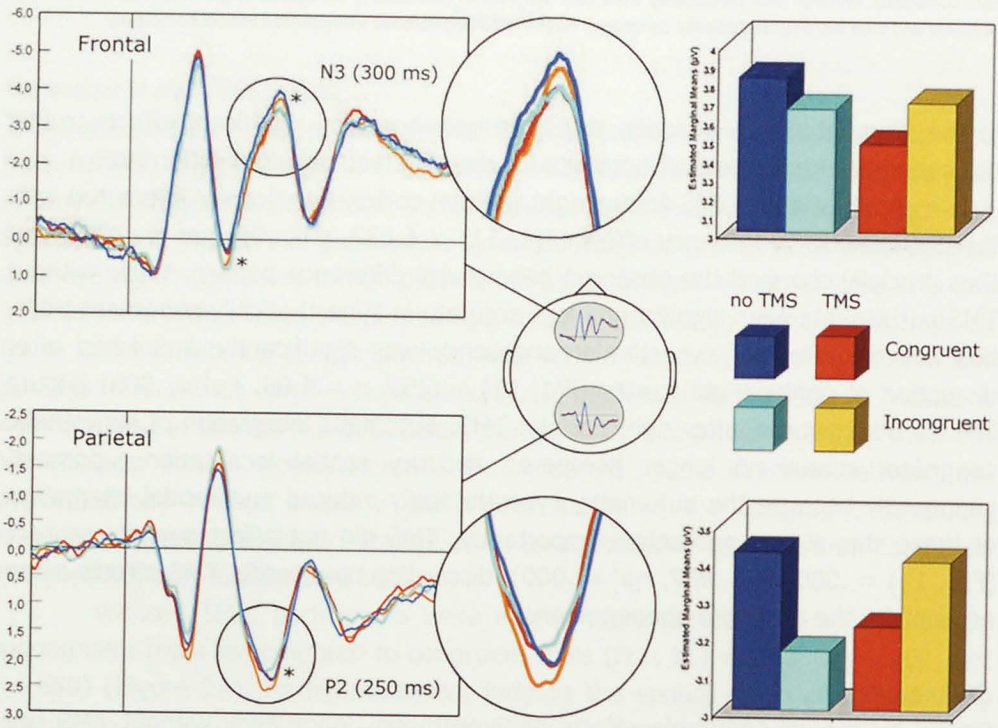

Figure 3. ERP results

ERP waves (-200, 800 ms) measured over frontal electrode AF3 (top panel) and parietal electrode CP6 (bottom panel) showing the significant interactions between synesthetic congruency and right parietal TMS. On the right estimated marginal means of individual frontal N3 and parietal P2 peak amplitudes.TMS reverses the effect of synesthetic congruency.

(P1, N1, P2 and N3), revealed that TMS most strongly modulated the P2 component recorded from parietal sites ($F(1, 9) = 12.147$, $p < .01$, $\eta p^2 = .603$). Increased positivity during synesthetically congruent as compared to incongruent trials was reduced after right parietal disruption with TMS (Figure 3). In addition, a very similar interaction between Synesthetic Congruency and TMS was observed in the N3 component recorded frontally around 300 ms after stimulus onset ($F(1, 9) = 6.249$, $p < .05$, $\eta p^2 = .439$) (Figure 3). A pattern of increased negativity during synesthetically congruent compared to incongruent trials was again reduced after TMS. Hence, disruption of right parietal cortex with TMS significantly influenced brain potentials recorded from parietal sites around 250 ms, and from frontal sites around 300 ms. The initially increased amplitude as a result of multisensory integration was diminished following right parietal TMS.

Besides this interaction effect, subsequent univariate analyses indicated a trend towards a Synesthetic Congruency effect in the N1 component at parietal recording sites around 200 ms after stimulus onset ($F(1, 9) = 4.744$, $p = .061$ $\eta p^2 = .372$), with higher amplitudes in synesthetically incongruent compared to congruent trials. In addition, P2 recorded from frontal sites revealed a significant Synesthetic Congruency effect ($F(1, 9) = 7.645$, $p < .05$, $\eta p^2 = .489$), consisting of an increased positivity during incongruent compared to congruent trials. The different amplitudes during synesthetically congruent and incongruent trials indicate that these two early components are associated with the automatic synesthetic integration, but in contrast to parietal P2 and frontal N3, they are not influenced by magnetic disruption of right parietal cortex. They are thus not reflecting the observed behavioural TMS-induced modulations. As the N1 component was not significant, but only indicated a trend, the link with pitch-size synesthesia is possibly also weaker than the significant parietal P2 component.

The similar interaction patterns between Synesthetic Congruency and TMS observed in parietal P2 and frontal N3 imply that these components are closely linked, possibly even originating from a single dipole. The same could be suggested for the Synesthetic Congruency main effects in parietal N1 and frontal P2. Indeed, Pearson product-moment analysis showed significant correlations between Parietal P2 and frontal N3 across conditions (Table 1A). This suggests that the parietofrontal P2-N3 is associated with the initial boost of multisensory integration caused by synesthetic congruency (as reflected in the reduced behavioural accuracy), and with the disruption of this integration by parietal TMS stimulation. Similarly, parietal N1 and frontal P2 were significantly correlated across conditions (Table 1A), implying a parietofrontal N1-P2 complex. Most likely, this complex also relates to synesthetic congruency, reflecting an earlier processing stage which was not modulated by TMS stimulation.

Correlations between behavioural and ERP results

Because TMS is an experimental manipulation of ongoing cortical processing, resulting modulations of both behaviour and event-related brain potentials can be causally attributed to the application of TMS. Following from this, we can hypothesise that certain TMS-induced changes in behaviour are reflected by certain

a			N1	P2	P2
No TMS	**Congruent**	**P2 parietal**	.167		
		P2 frontal	.928**	-.231	
		N3 frontal	-.300	.894**	.268
	Incongruent	**P2 parietal**	.226		
		P2 frontal	.927**	-.176	
		N3 frontal	-.331	.876**	.178
TMS	**Congruent**	**P2 parietal**	.445		
		P2 frontal	.867**	-.506	
		N3 frontal	.573	.659*	-.508
	Incongruent	**P2 parietal**	.490		
		P2 frontal	.941**	-.431	
		N3 frontal	-.346	.923**	.259

b	N1 parietal	P2 parietal	P2 frontal	N3 frontal
No TMS	-.103	-.583T	.062	.160
TMS	-.495	.648*	-.119	-.071

Table 1. Correlations between behaviour and ERP components

A) Within-subject correlations between the mean amplitudes of four relevant ERP components. Single asterisks indicate significance at the 0.05 level (2-sided), double asterisks indicate significance at the 0.01 level (2-sided). Parietal N1 amplitude significantly correlates with frontal P2 amplitude in all conditions. The same is observed for parietal P2 and frontal N3. B) Correlations between behavioural results and the mean amplitudes of four relevant ERP components. The asterisk indicates significance at the 0.05 level (2-sided), T indicates a strong trend towards an effect (p = 0.06, 2-sided). Since the interaction effects cannot be correlated directly, individual mean incongruent minus congruent accuracy scores are correlated with ERPs for no TMS and TMS separately. These results support the notion that the TMS-induced changes in the parietal P2 component directly account for the observed behavioural changes.

TMS-induced ERP modulations, allowing us to make direct inferences about the neural basis of a certain behavioural phenomenon. As described above, behavioural performance as well as the parietal P2 ERP component showed significant modulations as a result of synesthetic congruency, and were both significantly modulated by TMS over right parietal cortex. To confirm that the observed behavioural disruption of synesthetic multisensory integration – and thus pitch-size synesthesia as a whole - was indeed reflected in parietal P2, we computed Pearson product-moment correlation coefficients between behavioural performance and the four relevant ERP components. Indeed, a significant correlation was only revealed between behavioural performance and the parietal P2 component (Table 1B, Figure 4). Without TMS, there was a trend towards a negative correlation effect between Congruency and behavioural performance ($r = -.583$, $n = 11$, $p = .060$). After right parietal TMS, a significant positive correlation was observed ($r = .648$, $n = 11$, $p < .05$), suggesting that only the parietal P2 component, emerging around 250 ms, is functionally linked to the automatic integration of synesthetically congruent pitch-size stimuli.

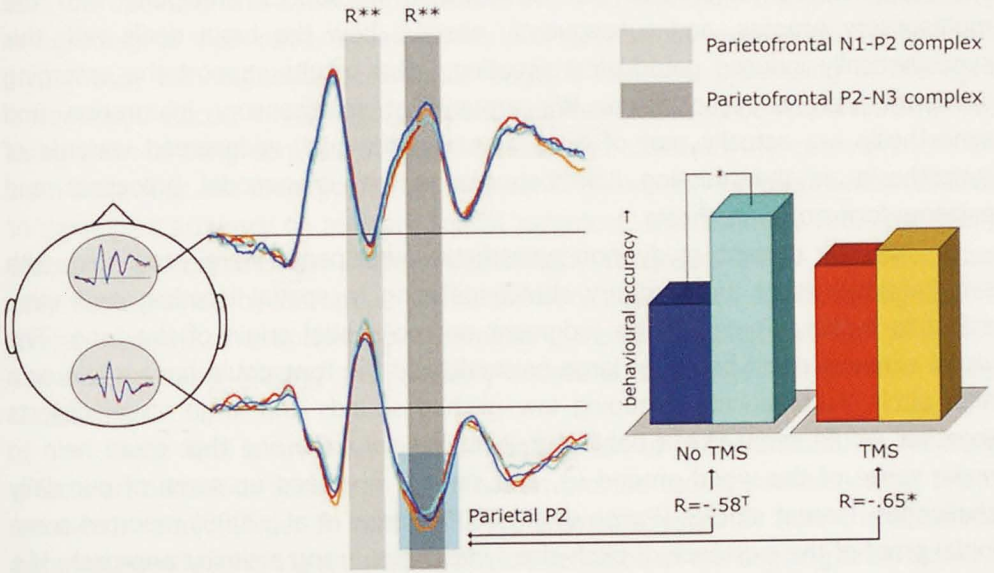

Figure 4. Correlations between ERPs and behaviour
Asterisks indicate statistical significance at the $p < 0.05$ level. Double asterisks indicate significance at the $p < 0.001$ level. Pearson product-moment correlation analysis revealed very strong correlations between parietal N1 and frontal P2, as well as between parietal P2 and frontal N3, indicating the existence of parietofrontal N1-P2 and P2-N3 complexes. However, only modulations in parietal P2 correlated strongly with the observed behavioural phenomenon of pitch-size synesthesia, without TMS as well as after magnetic disruption of right intraparietal cortex. This implies that especially the brain activity reflected in parietal P2 is causally linked to the observed behavioural effects. Modulations of the parietal P2 are able to reliably predict changes in behaviour, and provide a neural correlate of the observed TMS-induced changes at the behavioural level of pitch-size synesthesia.

Discussion

The current study investigated multisensory pitch-size associations, using a combination of psychophysics, transcranial magnetic stimulation and event-related potentials. Multisensory perception, or the process of accurately integrating or segregating incoming sensory events according to their mutual or separate origin, is usually governed by bottom-up stimulus characteristics, such as spatial and temporal correspondence of incoming sensory inputs. However, in case of ambiguity with regard to these bottom-up characteristics, the brain has to decide whether two stimuli belong to the same event, or not. We hypothesise that widespread, subtle forms of synesthesia provide crossmodal mapping patterns which underlie and influence multisensory perception on a large scale, but which especially become apparent when bottom-up features provide insufficient or contradictory information. Our aim was to investigate if such a mechanism plays a role in the case of pitch-size stimulus combinations. To this end we employed a pitch-size variant of the spatial Ventriloquist illusion. By introducing this paradigm we could test whether pitch-size synesthesia introduces ambiguity into the multisensory process, and subsequently observe how the brain deals with this synesthetically induced paradoxical situation. Our results support the emerging spectrum view of synesthesia. We argue that multisensory integration and synesthesia are actually part of a continuum, with mild, widespread variants of synesthesia as the 'missing link' between normal crossmodal processes and extreme forms of synesthesia.

In the current study non-synesthetic participants were presented with simultaneous visual and auditory stimuli differing in spatial location, and were asked to make a forced-choice judgment on the spatial origin of the tone. The visual stimulus could be either large or small, and the tone could have a low or a high pitch. Automatically grouping low pitched sounds and large visual objects together would seem like a behaviourally beneficial trait, one that could help to make sense of the world around us, and simplify or speed up some of our daily challenges. Recent studies (Parise et al., 2008; Parise et al., 2009) reported some initial proof of the existence of pitch-size synesthesia, using a similar approach. If a process is deeply rooted into behaviour, it will still be carried out if this results in decreased performance. We assumed that if low pitched sounds are indeed matched with large visual stimuli, and vice versa, these synesthetically congruent objects would be grouped together by brain structures involved in multisensory integration, despite the fact that the objects actually originated from different spatial locations. The spatial origin of the sound would be pulled towards the location of the visual stimulus. Judging the spatial origin of the auditory stimulus

would become more difficult because of the brain's effort to ascribe the congruent perceptions to a single source. This is exactly what we observed: behavioural performance with regard to judging the spatial origin of a sound was decreased when sound and visual object were synesthetically congruent. This supports that pitch-size synesthesia is a widespread trait in the normal population. In addition, the fact that this integration occurred even in a situation in which it is actually detrimental to performance, i.e. while the participants were aiming at keeping the stimuli segregated to localise the sound, implies that the process is compulsory and highly automatic.

Based on these psychophysical data we can confirm that sounds with a certain pitch are automatically bound with visual objects with a certain size, even if one of the basic rules of integration, spatial correspondence, is violated. This is an example of crossmodal integration which cannot be explained solely by simple bottom-up features of the stimuli. In contrast, it seems that there is an underlying mapping of pitch-size associations. We argue that this underlying mapping constitutes a subtle form of synesthesia, common in the normal population, and that disruption of this synesthetic process will also inevitably disrupt multisensory integration, or vice versa, because they are qualitatively equivalent. To test this hypothesis, we magnetically disrupted ongoing neuronal processing in the right intraparietal sulcus, a higher perceptual brain area known to be responsible for the integration of congruent multisensory inputs, and observed the consequences on both behaviour and brain potentials. Without TMS, synesthetic congruency resulted in decreased accuracy on auditory spatial judgment. Interestingly, TMS specifically increased performance on those synesthetically congruent trials. This effect can only be explained by disruption of multisensory integration: top-down signals no longer overrule the bottom-up information, thereby wiping out the pitch-size synesthesia, and leaving the auditory locations unchanged and more easily localisable.

Since TMS is an experimental manipulation of brain function, its resulting effects on behaviour and brain potential can provide causal information on brain-behaviour relationships. In this case, we were interested in how the observed behavioural effects of right parietal TMS are reflected in the ongoing brain activity. The differential event-related brain potentials with regard to synesthetically congruent and incongruent trials observed in the parietal N1 component, which are significantly correlated with a similar pattern in subsequent frontal P2, could point in the direction of an early synesthetic effect. The increased amplitudes during incongruent trials in the parietofrontal N1-P2 complex might reflect enhanced discriminative or distorted categorisation processes (Vogel & Luck, 2000), which speaks to a combined representation of the congruent stimuli already at an early

stage of processing. However, since this component only shows an influence of synesthetic congruency, and no influence of TMS, it cannot account for the improved auditory spatial judgment in synesthetically congruent trials after TMS. The low correlation between frontal N1 and behavioural results further supports this notion.

The subsequent P2-N3 complex, on the other hand, shows significant interaction effects between congruency and TMS, and moreover, in a pattern that is comparable with that observed in the behavioural data: as a result of TMS, the difference in brain potential amplitude between congruent and incongruent trials is reduced, even reversed. This effect is first visible in the parietal P2 component around 250 ms, and subsequently in the frontal N3 component around 300 ms. Although significant correlations between parietal P2 and frontal N3 suggest a parietofrontal P2-N3 complex possibly originating from a single dipole, the significant correlation between behavioural results and parietal P2, but not frontal N3, indicates that especially the brain activity reflected in parietal P2 is causally linked to the observed behavioural effects. Apparently, modulations of the parietal P2 are able to reliably predict changes in behaviour. Considering that TMS is an external manipulation of brain function, it seems safe to conclude that TMS over the right intraparietal sulcus diminishes the Ventriloquist illusion arising from synesthetically congruent pitch-size combinations, which is reflected most strongly in the parietal P2 ERP component around 250 ms after stimulus onset.

We can conclude that multisensory integration plays an important role in pitch-size mapping, a widespread and adaptive form of synesthesia. These results support the increasingly cogent view of synesthesia as part of a spectrum ranging from normal crossmodal processes, via subtle and prevalent forms of synesthesia, to the curious, more extreme forms that have attracted most scientific attention so far. Currently, two competing theories on the neural substrates of synesthesia are under debate. According to these theories, synesthesia is either ascribed to excess anatomical connections between low-level sensory areas, which are normally pruned in development (Hubbard et al., 2005a; Rouw & Scholte, 2007; Weiss et al., 2005), or to a disinhibition of feedback from higher perceptual to unisensory areas (Grossenbacher & Lovelace, 2001; Mulvenna et al., 2006). Until hitherto empirical research on this topic has not been able to clearly dissociate between these two theoretical accounts for the phenomenon of synesthesia. Whereas Cohen Kadosh and colleagues (Cohen Kadosh, Henik, Catena et al., 2009) were able to induce grapheme-colour synesthesia in otherwise non-synesthetic participants using posthypnotic suggestion, supporting the disinhibited feedback theory, Rouw and colleagues uncovered increased anatomical connections in synesthetes (Rouw et al., 2007), which furthermore differed between projectors

and associatiors (Rouw et al., 2010). With regard to our current results, the observations that TMS over a higher order, crossmodal region like the right intraparietal sulcus crucially interfered with the synesthetic process, and that ERP data reflect modulations in parietal and subsequently frontal sites at relatively long latencies of around 250 ms, speak more to the disinhibited feedback theory than to the excess anatomical connections theory.

In conclusion, although pitch-size congruency can be considered an adaptive trait, it hampers performance in an auditory localisation task as a result of the Ventriloquist illusion: synesthetic congruency results in multisensory binding, and consequently auditory sources are misallocated to the visual source. Transcranial magnetic stimulation over right intraparietal sulcus, a known multisensory integration site, is able to wipe out this Ventriloquist effect, and specifically improve performance on synesthetically congruent trials. This experimental manipulation is reflected in the parietal P2 component, which correlates strongly with behavioural performance both with and without TMS. The current results confirm that pitch-size synesthesia is a common trait within the normal population, and that normal multisensory processes play a crucial role in this process, thus supporting the increasingly cogent spectrum view of synesthesia. A multi-methodological approach, investigating the effects of magnetic disruption of specific cortical processes on both recordings of event-related brain potentials and behavioural measures of performance, enabled us to provide detailed information about the cortical components and temporal aspects of this process. However, this in itself does not fully solve the debate about whether synesthesia is an isolated, curious phenomenon, or whether it is part of a continuum shared with normal multisensory processes, with subtle synesthetic processes as the yet unacknowledged missing link. Whether to call these effects 'crossmodal-plus' or 'synesthesia-light' is purely a matter of semantics. We suggest that future studies should aim at further exploring these processes and their interaction with multisensory perception, as well as their link with more extreme forms of synesthesia.

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Chapter 5

Combining theta burst patterned TMS and event-related fMRI to visualise cortical compensational mechanisms during spatial mental imagery

Based on:
Bien, N., Goebel, R., and Sack, A.T. (in preparation). Combining theta burst patterned TMS and event-related fMRI to visualise cortical compensational mechanisms during spatial mental imagery

Abstract

Spontaneous brain plasticity occurring after a brain is lesioned can contribute greatly to patient rehabilitation. Yet, the mechanisms underlying this process are still elusive. We aimed to reveal first direct insights into the neuronal mechanisms underlying instantaneous compensatory brain processes, which supposedly arise to bypass a TMS-induced virtual lesion, in order to maintain behavioural performance. We combined long-lasting TMS over left or right parietal cortex with subsequent fMRI, during which a spatial mental imagery task was executed. Using a new, parametrically varied version of the mental clock task, we were able to for the first time top-down dissociate between mental imagery and subsequent spatial angle comparison stages of the task. Our results show that whereas mental imagery recruits frontoparietal networks bilaterally, angle comparison processing remains largely confined to the right intraparietal sulcus. Subsequently, by contrasting fMRI data acquired after cortical disruption with long-lasting TMS, we showed that specifically after left parietal TMS instantaneous compensational processes are set in motion, recruiting bilateral parietal but, surprisingly, most strongly ipsilateral frontal cortex. In addition, a large increase in compensational activation related to the spatial angle comparison phase of task processing was observed throughout the functional network initially already involved in the mental clock task, but most strongly in left parietal regions surrounding the site of the virtual lesion. After right parietal TMS no such compensational effects on brain activation were observed. However, due to an unexpectedly strong learning effect masking potential TMS effects on behavioural performance, is it currently not clear whether this lack of activation indeed reflects a failure to compensate for the virtual lesion, or whether it reflects a general lack of effect of TMS at the behavioural level.

Introduction

Although brain plasticity is a term usually associated with infancy and development, the adult brain is not as static and invariable as is still often believed. Throughout the course of life, any brain will be subjected to different external and internal influences and changing demands, and flexibility in coping with such influences constitutes a clear evolutionary advantage. As the famous psychologist William James shared great insights about almost any topic of psychology, the topic of brain plasticity is no exception: "Plasticity [...] means the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once. Each relatively stable phase of equilibrium in such a structure is marked by what we may call a new set of habits. Organic matter, especially nervous tissue, seems endowed with a very extraordinary degree of plasticity of this sort..." (William James, 1890, The Principles of Psychology, Volume I, p. 105). One of the most obvious and frequent examples of brain plasticity is learning. Whether one is learning to speak, learning to drive a car, or learning how to work with a new software package, learning is inevitably accompanied by plastic changes in the brain which promote preservation of the learned facts and skills for future application. This process is so natural and self-evident that it is taken for granted.

However, there are situations in which the need for a flexible brain suddenly becomes more obvious, for example when the brain is damaged, either by externally induced trauma or by internal catastrophes such as stroke. After the brain has been lesioned, patients suffer, to a smaller or larger extent, from debilitating behavioural, cognitive and/or emotional problems. In Europe alone, over 1.8 million patients are estimated to suffer from the results of stroke or brain trauma, which costs European society 25 to 45 billion Euros annually (Andlin-Sobocki, Jönsson, Wittchen, & Olesen, 2005). In addition, the average age of patients suffering from stroke is continuously decreasing. Hence, optimising treatment and rehabilitation of brain lesions is a pressing matter. From decades of lesion studies it has become clear that damage inflicted on functional neural networks will either lead to behavioural impairment, or to compensatory processes and thus new patterns of brain activation in order to sustain behaviour. Accordingly, a subset of patients experience partial functional recovery of lost abilities, especially during the first weeks and months after lesion onset. Exposing which neuronal mechanisms underlie these spontaneous recoveries and which factors determine the amount of spontaneous recuperation in individual patients, would not only provide greatly advanced insights, but more importantly it would also pave the road towards using these insights to improve functional restoration in lesion patients. To date, such an effective treatment for rehabilitation is still

missing, mostly due to a general lack of knowledge regarding the underlying neuronal mechanisms governing brain plasticity.

Despite the obvious importance of studying brain plasticity, the mechanisms underlying this process are still elusive, which is at least for an important part due to the methodological difficulties associated with studying it. As has been described in the introductory chapter, although studies on lesioned patients have in the past contributed enormously to the understanding of brain-behaviour relationships and the functional layout of the brain, they are also inevitably methodologically flawed in many aspects. In the case of brain plasticity, investigations would have to take place during the acute phase of a lesion, preferably as early as possible. However, understandably, patients suffering from the acute consequences of a lesion have other things to worry about, and might not be inclined to subject themselves to systematic scientific investigations. In addition, three important confounds would remain. The size and anatomical location of the lesion would differ between patients, the composition of the sample with regard to age, sex, health and other possible confounding factors would be beyond the control of the researcher, and since patients at the earliest only seek treatment from when the lesion has occurred, it would be impossible to objectively infer pre-lesion behavioural and cognitive states.

In this light, a more elegant and efficient tool to investigate brain plasticity under controlled experimental conditions is transcranial magnetic stimulation (TMS). TMS allows for non-invasive direct local interference of cortical processing in a certain brain area, thus temporarily creating a 'virtual lesion' in conscious healthy volunteers. Observation of resulting behavioural changes provides causal information about the functional relevance of a certain brain area. With this virtual-lesion approach of TMS, healthy volunteers can temporarily be turned into neurological patients – and back into healthy adults under controlled experimental conditions (Sack, 2010). This allows for random selection of 'patients', and importantly it also permits within-person balanced comparisons between behaviour observed with and without the virtual lesion. For almost two decades, scientists and clinicians have now used the behaviour-modulating capacity of magnetic brain stimulation to reveal causal brain-behaviour relationships, investigating an ever increasing range of different cognitive functions. However, in the case of functional compensation, behavioural performance would be fully or partially preserved on account of an instantaneous reorganisation. In any case, if compensation would take place, the mechanisms underlying it would not become evident from the behavioural data. Contrarily, functional imaging of brain activation patterns both during the compensation phase and during phases in which no compensation is taking place, would potentially reveal these processes.

In the current study, we combined the virtual lesion approach of TMS with the visualisation of brain activation patterns using fMRI, to gain insight in instantaneous reorganisational processes taking place after magnetic disruption of cortical processing. We did so in a setting of spatial mental imagery, since evidence has previously been provided that compensational processes might take place during this procedure to preserve performance after TMS (Sack et al., 2005; Sack et al., 2002). We employed the mental clock task (MCT), a well-investigated mental imagery task which involves spatial judgment of two angles, and produces controllable behavioural output (Trojano, Grossi, Linden et al., 2000). The task is simple, but requires complex and quite lengthy cognitive and cortical processing: two clock times are presented auditorily, and the participant is then asked to generate mental images of these clocks, compare which of the angles formed by the small and large hands of each clock is larger, and indicate the result by pressing a button. Previously, the MCT was shown to involve activation of bilateral posterior parietal cortices (PPC) (Trojano et al., 2000).

In an extended investigation of this task, Formisano and colleagues (2002) showed a more widespread bilateral network of brain areas involved, including PPC, dorsolateral prefrontal cortex (DLPFC) and supplementary motor area (SMA). In addition to showing this network, they were also able to infer the different temporal stages during which these areas contributed to task execution, by visualising how blood oxygen level dependent (BOLD)-activation progressed through the cortex from the onset of the auditory stimulus until the final behavioural response of each experimental trial. Despite the sluggishness of the hemodynamic BOLD-response, which takes about 4 to 5 seconds to reach its peak, the relatively long and complex cortical processing required to correctly fulfil an experimental trial of the MCT allowed for this procedure. Activation spreaded from the auditory cortex, through DLPFC and SMA, to PPC, and finally reached motor cortex. Whereas the auditory and motor cortex activations can be directly retraced to the auditory stimulus and the final button press, respectively, DLPFC, SMA and PPC seem to be involved in the intermediate stages of the task: mental imagery of the two clocks, and subsequently the spatial comparison of the two angles. While DLPFC, SMA and PPC seem to be largely co-activated during a longer period of time, a hemispheric asymmetry is observed in the parietal cortex: left PPC seems to be activated earlier in the process than part of the right PPC, indicating a functional distinction between the two. This hypothesis was further strengthened by subsequent correlational analyses between single-trial estimations of onset, width and amplitude of the BOLD signal in different brain areas, and the reaction time on the same trials. Whereas in DLPFC, SMA and left PPC the width of the BOLD correlated with the reaction time, in right PPC a correlation between BOLD

onset and reaction time was observed. Based on this information, the authors inferred that left PPC is involved in a process starting relatively early after stimulus onset, the duration of which is of influence on the reaction time. The mental imagery stage of the task would best fit this shoe. The BOLD signal in the right PPC, on the other hand, has a relatively late onset, which is correlated with reaction time. The authors inferred that right PPC is involved in a process which only starts after earlier processing has been completed, and the completion of which is crucial for the response time. This fits best with the spatial angle comparison stage of the MCT. Hence, it seems that left and right PPC might be responsible for different, sequentially executed functional stages of the MCT.

In a follow-up of this study, this notion was subjected to further testing by Sack and colleagues (2002). They used the virtual lesion approach of repetitive TMS (rTMS) to distort cortical processing in left or right PPC prior to MCT execution. Interestingly, after right PPC participants displayed a slower response to the MCT, whereas after left PPC no behavioural impairment was observed. Hence, although bilateral PPCs showed increased task-related BOLD activation in Formisano's previous fMRI study, only magnetic disruption of right PPC also led to significant behavioural impairment. This observation further strengthened the supposition of an asymmetrical functional distribution across left and right PPC during spatial mental imagery. As the authors postulated, right PPC might possess the ability to take over the functions normally carried out by left PPC, thus instantaneously compensating for functional disruption of left PPC, for example after TMS. The other way round, left PPC would not be able to compensate for a loss of function in right PPC, which would explain why TMS over right PPC did result in behavioural impairment.

To test this hypothesis of right PPC being able to compensate for loss of function in left PPC but not vice versa, Sack and colleagues (2005) combined rTMS with triple pulse TMS (tpTMS) time-locked to task execution. While left PPC was either disrupted using rTMS or left undisturbed, right PPC was disrupted at different time points relative to stimulus onset. Without prior rTMS over left PPC, right PPC tpTMS resulted in behavioural impairment in a relatively late time window. However, after rTMS over left PPC, tpTMS over right PPC disrupted behavioural performance in an additional earlier stage of MCT execution, further strengthening the postulation that right PPC could take over the functions normally fulfilled by left PPC at an earlier stage of the task.

Hence, there is strong evidence that compensational processing might indeed play a role after virtual lesions have been inflicted on parietal cortex, and that, at least during spatial mental imagery, there is a right hemispheric dominance with regard to these compensational abilities of parietal cortex. Consequently, only

after right PPC has been virtually lesioned, behavioural performance is compromised. This evidence is in line with a longstanding tradition of lesion studies, which consistently show that disabilities with regard to spatial attentional processing are usually confined to the left hemifield, arising after right parietal cortex has been damaged (Vallar et al., 1986). Nevertheless, these compelling conclusions regarding the dynamic neuronal plasticity of the adult human brain are still only indirectly inferred, based on TMS-induced changes in behavioural performances.

As a result, it still remains unclear by which neuronal mechanisms these behavioural consequences of TMS – or more importantly, the lack thereof - and the dynamics of brain plasticity are mediated in the brain. The only way to overcome this issue would be to directly assess neurophysiological measures of the behavioural effects induced by TMS. In the current study, for the first time, the cortical consequences of virtual lesions to left and right PPC were assessed, by applying a recently developed, long lasting patterned rTMS protocol called theta burst stimulation (TBS) (Huang et al., 2005), and immediately afterwards visualising the effects of these lesions both on behavioural performance and on cortical activation patterns using fMRI. We specifically aimed to visualise the neuronal consequences of virtual brain lesions, and to reveal first direct insights into the neuronal mechanisms underlying compensatory brain processes used to bypass a virtual lesion in order to maintain behavioural performance. Based on previous results we hypothesised that a TMS induced virtual lesion to the left PPC might evoke instantaneous functional reorganisation of brain activity in an attempt to maintain behavioural performance during spatial mental imagery, whereas this effect would not be observed after right PPC disruption.

While employing the MCT, we added a new feature to this well-established paradigm, which allowed us for the first time to dissociate between the mental imagery and spatial angle comparison stages of the spatial mental imagery process from a top-down perspective. By parametrically varying the amount with which the angles of the clock hands - representing the two clock times in each trial – differed, we were able to dissociate between mental imagery and spatial comparison processes (Figure 1). While the spatial relation between the two angles would not be of any influence yet during the mental imagery phase, a smaller difference was hypothesised to result in increased task difficulty from the spatial comparison phase onwards. This parametric increase of task demands with smaller angles would supposedly not only result in longer response delays, but importantly also in a similar difficulty-related modulation of BOLD-response in those brain regions preferentially occupied with the spatial comparison of the angles. Based on

the previously discussed existing evidence, this parametric modulation related to angle comparison would be expected to emerge at right PPC.

Methods

Participants

12 neurologically healthy volunteers with normal or corrected to normal vision (mean age 24 ± 2.2), 5 of which were male, participated in this study. All participants were unaware of the goal of the study until after having completed their participation. Before the start of each experimental session, each participant provided written informed consent and was screened for TMS and fMRI experimentation safety by an independent medical supervisor. Ethical approval was given by the local medical ethical committee. Participants were rewarded with gift certificates or student participation credits.

Experimental design

The Mental Clock Task (MCT), a well-known spatial mental imagery task, was employed throughout this study (Figure 1). Each trial of this task started with the auditory presentation of two clock times, e.g. "two... four-thirty", vocalised by a male voice. Participants were requested to first create visual mental images of analogue clocks displaying the respective times. Subsequently, they were required to estimate the spatial angles between the small and large hands of each of the clock faces, and compare them. If the hands indicating the first time formed the greater angle, they were asked to press a button with their left index finger. If the

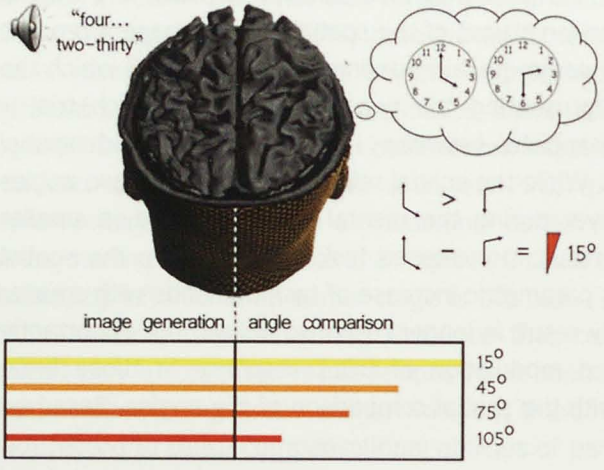

Figure 1. Adapted mental clock paradigm In the mental clock task (MCT) participants are required to mentally image two analogue clocks depicting aurally presented times, compare the angles between the hands of each clock, and decide which clock has te bigger angle. An adapted form of the MCT was employed, in which parametrically varying differences between the angles of the two mentally imaged clocks resulted in four levels of task difficulty. The current design allowed for top-down dissociation of brain activation patterns between two functional stages of the MCT; mantal image generation versus angle comparison.

second time formed the greater angle, they responded with their right index finger. In the current study we added a new feature to this established mental imagery task, in the form of a parametric variation in difficulty. In each stimulus, consisting of a unique combination of two times, the difference between the angles formed by the respective hands of the clock could differ by either 15, 45, 75, or 105 degrees. We hypothesised that in case of two angles differing by only 15 degrees it would be quite difficult to judge which time had the greater angle, resulting in a slower response, whereas this difference would be more obvious when the angles differed by as much as 105 degrees. In addition, we hypothesised that it is only after the stage of mental image formation has been completed, when the spatial angles of the two clocks have to be compared, that this level of difficulty becomes relevant for performance speed. After all, during imagery of two analogue clock faces it is irrelevant how these two faces relate to each other. This added feature of different difficulty levels has the potential to disentangle brain areas which do not show parametric variations of the BOLD, and which are thus most likely involved in stages up to the mental imagery phase, from brain areas showing specific parametric hemodynamic modulations, which are most likely relevant during the spatial angle comparison process. Furthermore, if TMS over left or right parietal cortex differentially targets certain functions and phases of task processing, as postulated by previous studies, this would become evident from differential modulations of the hemodynamic response.

The study consisted of 3 experimental scanning sessions of 1 hour each. During each session two functional runs during which participants performed the MCT were recorded, as well as a high resolution anatomical scan. In 2 out of the 3 sessions, theta burst TMS was applied directly prior to scanning (see section TMS design and apparatus for details), either over left (TMS-L session) or over right (TMS-R session) parietal cortex. Prior to the third session no TMS was applied (no TMS session). The order of sessions was counterbalanced across all 12 participants. If the randomisation scheme implicated that the first session would involve TMS, a short localiser scanning session was conducted prior to this first experimental session, in order to provide the brain data necessary for functionally neuronavigating the TMS coil position over left and right parietal cortex (see section TMS design and apparatus for details). During each experimental session functional brain activation data were recorded, as well as psychophysical data (reaction times and accuracy).

Stimulus material

Throughout the study, Presentation software (Neurobehavioural Systems, Inc., Albany, USA) was used for both stimulus presentation and recording of the

behavioural responses. Each auditory stimulus had a duration of 2000 ms. Clock time combinations were balanced for difficulty level (15, 45, 75 or 105 degrees difference), clock face (left, right or bilateral) and response hand (left or right). Only whole (e.g. 'two') and half (e.g. 'four-thirty') hours were included, and times with a 90 degree angle between the clock hands were left out. In total, 48 unique combinations of clock times were formed.

TMS design and apparatus

Directly prior to the start of 2 out of the 3 imaging sessions, continuous theta burst TMS (cTBS) (Huang et al., 2005) was applied over either left (TMS-L session) or right (TMS-R session) posterior parietal cortex. cTBS is a patterned TMS protocol, in which 600 pulses administered over a period of 40 seconds result in local cortical inhibition lasting for approximately one hour (Huang et al., 2005). These long lasting effects of TBS make it a particularly suitable inhibitive TMS protocol to allow for subsequent imaging of the neural effects of TMS. cTBS was applied at 80% of the resting motor threshold of each individual participant, which was assessed at the start of the first TMS session. A Medtronic MagPro X100 stimulator (Medtronic Functional Diagnostics A/S, Skovlunde, Denmark) was employed. Magnetic pulses were delivered with a handheld standard figure-eight-coil (Magnetic Coil Transducer MC-B70, Medtronic).

Stimulation locations in the left and right parietal cortex were identified by performing independent component analysis (ICA) on one functional run of the MCT, which was recorded either during the first experimental session (in case this session was without TMS according to the randomisation scheme) or during a separate localiser scanning session. A right lateralised parietal cluster provided the right parietal stimulation site, whereas the left counterpart of a bilateral parietal cluster was taken as the left parietal stimulation site (Figure 2). Participants were coregistered to their structural brain imaging data using the BrainVoyager TMS Neuronavigator (Brain Innovation BV, Maastricht, the Netherlands), a stereotaxic frameless neuronavigation system which allows for continuous online coil navigation directly above a cortical target point localised using fMRI activation. TMS target points were defined on the reconstructed cortical surface using individual functional brain imaging data.

Figure 2. TMS target sites
The TMS stimulation sites of each of the 12 participants was individually defined based on clusters arising from independent component analysis. The left stimulation site was part of a bilateral posterior parietal cluster, whereas the right stimulation site was defined from a unilateral right cluster of activation. Clusters showing individual stimulation clustera are shown superimposed on segmented hemisphere, clolour coded for individual participant identity.

fMRI design and apparatus

All three fMRI sessions were identical in design, except for the presence or absence of TMS preceding the imaging session. A slow event-related design was employed. Two functional runs containing 48 mental clock trials each were acquired with a repetition time (TR) of 1000 ms. Total trial duration including stimulus presentation, processing, response and an intertrial interval was 16, 17, or 18 seconds/volumes. Total run duration was 14 minutes.

Brain imaging data were gathered using a 3-T Siemens Allegra magnetic resonance (MR) scanner and a volume head coil at the Maastricht Brain Imaging Centre (M-BIC) at Maastricht University, the Netherlands. Functional images were acquired using single-shot gradient-recalled echoplanar imaging (840 volumes, TR = 1000 ms, echo time (TE) = 28ms, flip angle = 68°). Eighteen oblique slices, positioned over the parietofrontal regions of the brain, were acquired with a 0.5 mm interslice gap. The field of view (FOV) was reduced to 210 to allow for smaller voxel size. Voxel size was 3.2 x 3.2 x 2.5 mm^3 , and each slice contained a matrix of 64 by 64 voxels. High-resolution (voxel size 1 mm^3) full-brain 3D anatomical data were collected using a T1-weighted ADNI sequence.

Auditory stimuli were presented through MR compatible headphones. A black background with a white fixation cross was continuously displayed onto a frosted screen, positioned at the rear end of the scanner bore, using an LCD projector (PLC-XT11-16, Sanyo North America Corporation, San Diego, CA). An adjustable mirror mounted on the head coil allowed each subject a complete view of the display. Responses were recorded using a standard MR compatible button box (LUMItouch keypads, Photon Control, Burnaby, Canada).

Behavioural and TMS data analysis

Reaction times and accuracy of responses were recorded during brain imaging. False responses and outliers were removed from further analysis.

To first assess the effect of the hypothesised modulation of difficulty by parametrically varying the difference between the angles of the two mental clocks, Repeated Measures ANOVA procedures were carried out on response times and accuracy values obtained during the 'no TMS' baseline session, with the degrees of difference between the two times of the clocks (15, 45, 75 or 105 degrees difference between the hands of the first and second clock time) as a single factor. Subsequent statistical analysis of the reaction times recorded during the fMRI session consisted of a 3 by 4 Repeated Measures ANOVA, containing the factors TMS stimulation site (no TMS, TMS-L, or TMS-R) and degrees of difference between the two times of the clocks (15, 45, 75 or 105 degrees). Again, a similar analysis was carried out with the accuracy values as a dependent variable. In case of significant main effects, post-hoc simple comparisons were carried out. Alpha values of pairwise comparisons were Bonferroni corrected to avoid the multiple comparison problem. The degrees of freedom of the error variance were Greenhouse-Geisser corrected to anticipate sphericity issues.

Figure 3. Coverage of functional voxels preserved for group analysis in volume space
Although each individual anatomical and functional dataset was spatially standardised according to Talairach procedure, functional volumes obtained from different functional runs never fully overlap, even more so in case partial brain coverage is employed. Since only functional voxels which are represented in each individual functional run (green voxels in the figure) are taken into account for GLM analysis, volume-space group analysis results in massive loss of functional voxels. This is most severe in the ventral slices, and at the outer boundaries of the acquisition space, which represent the grey matter which contains the functional signal of interest. Especially posterior parietal grey matter voxels were affected. To tackle this issue, reconstructed cortical surfaces of each individual participant were entered into a cortex-based alignment procedure. Besides optimising anatomical alignment, this procedure also ensured that the grey matter voxels, which are containing the paramount functional information, of each individual participant are entered into the group analysis.

fMRI data analysis

Functional and anatomical brain imaging data were pre-processed and analysed offline using BrainVoyager QX (Brain Innovation BV, Maastricht, Netherlands).

The first 8 volumes of each run were excluded to allow for T1 saturation, permitting the T2-weighted MR signal to stabilize. The first functional volume served as a high-contrast prototype to which following functional volumes were aligned during pre-processing. Pre-processing of the functional data existed of slice scan time correction using sinc interpolation, 3D motion correction involving trilinear-sinc interpolation, linear trend removal, and application of a 3 cycles per timecourse high pass filter. Individual functional runs were inspected visually to ascertain that subjects had not moved excessively during data recording, which was not the case. Anatomical data were standardised using Talairach transformation (Talairach et al., 1988). Functional slices were coregistered to the anatomical data on the basis of positioning parameters obtained from the scanner, and subsequent manual adjustments to ensure optimal fit. Subsequently, the 2D functional runs were transformed into Talairised 3D volume timecourses, using sinc interpolation. Volume time courses were spatially smoothed using a 3 mm FWHM kernel. Anatomical data from the three or four scanning sessions were averaged, and subsequently segmented at the gray-white matter boundary using a semiautomatic procedure based on intensity values, as implemented in BrainVoyager QX. For group analysis, cortex-based alignment (Goebel, Esposito, & Formisano, 2006) was used to optimise spatial matching of cortical locations between participants, but especially to prevent massive exclusion of functional voxels from the subsequent GLM data analysis due to not fully overlapping functional slices (Figure 3).

Statistical analysis of the variance of the blood oxygen level-dependent (BOLD) signal was based on the application of multiple regression analysis to time series of task-related functional activation (Friston et al., 1995). Dummy coding was employed in order to allow for extrasession comparisons. Analysis was performed over volumes acquired between stimulus onset and the final button response, whereas fixation periods between the button response of one trial and stimulus onset of the next were used as a baseline. Predictor time course were adjusted for the hemodynamic response delay by convolution with a hemodynamic response function (Boynton et al., 1996). Contrasts between conditions were carried out using BrainVoyager's Random Effects (RFX) GLM analysis tool, enabling generalisation of the statistical inferences to the population level. For visualisation purposes, statistical activation maps were projected onto an inflated reconstruction of the cortex-based aligned average brain of all 12 participants.

In addition to standard GLM analysis of the functional timecourses, parametric modelling was employed in order to identify brain regions which were specifically modulated by trial difficulty and thus, as hypothesised, were more likely to be involved in the spatial comparison of the two angles. Each trial was weighed according to its difficulty level: trials with the smallest difference between their angles (15 degrees) received a weight of 4, trials with 45 degrees were weighed as 3, trials with 75 degrees as 2, and the easiest condition with the largest difference (105 degrees) received the standard weight of 1. RFX GLM analysis was carried out with the factor TMS (no TMS, TMS-L or TMS-R), and produced statistical activation maps for main effects, as well as for parametric effects. To visualise brain areas which showed a main effect of the task, as well as a parametric modulation, a conjunction contrast involving both was carried out (main effects $\wedge$ parametric effects). To test whether TMS differentially modulated this parametric modulation of cortical activation, contrast maps of these conjunctions were produced, comparing TMS-L and TMS-R to no TMS.

Results

Behavioural and TMS results

Firstly, the effect of parametrically varying the difference between the angles of the hands of the two mentally imaged clocks was assessed, to test our hypothesis that a smaller difference would increase the difficulty of task processing, and thus increase response times. Repeated Measures GLM analysis over the response times showed a significant main effect of difference in degrees ($F(3, 9) = 11.5$, $p = 0.002$), as well as a significant linear ($F(1, 11) = 27.2$, $p < 0.001$) and quadratic contrasts ($F(1, 11) = 19.1$, $p = 0.001$). Pairwise comparisons confirmed that participants responded significantly slower in trials with 15 degrees difference, compared to each other difference level ($p = 0.002$, $p < 0.001$, and $p = 0.002$, respectively) (Figure 4a). Analysis of behavioural accuracy showed similar results: a significant main effect of difference in degrees ($F(3, 9) = 7.2$, $p = 0.009$), as well as the significant linear ($F(1, 11) = 21.1$, $p = 0.001$) and quadratic contrasts ($F(1, 11) = 12.2$, $p = 0.005$). Again, pairwise comparisons confirmed that participants had a decreased accuracy on trials with 15 degrees difference, compared to each other difference level ($p = 0.013$, $p = 0.008$, and $p = 0.004$, respectively) (Figure 4b). These findings corroborate our hypothesis about varying task difficulty by parametrically varying the difference between the two mental clocks, and warrants subsequent treatment of this factor as a difficulty manipulation reflecting spatial angle comparison processes. Furthermore, the

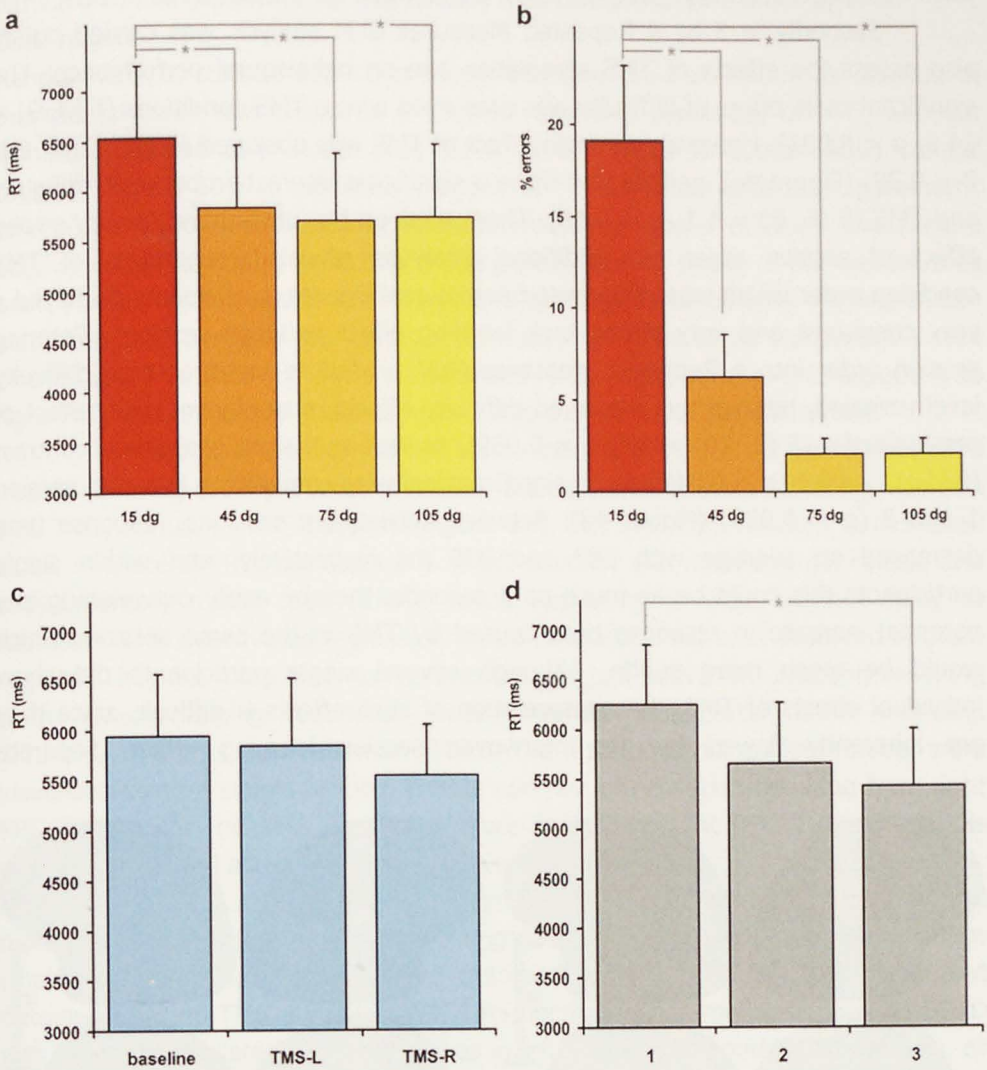

Figure 4. Behavioural results

a) Reaction times (ms) for the four levels of difference between the clock times. The most difficult condition (15 degrees difference between the angles representing the two clock times) differs significantly from the other three conditions. This confirms the hypothesis that a parametric modulation of angle differences results in differences in task difficulty. b) Similarly, error rates in the condition with the smallest difference in degrees are significantly higher than in the other three conditions, ruling out speed-accuracy trade-off accounts as an alternative explanation for the difficulty effect observed in the reaction times. c) No significant effects of TMS on the reaction times are observed. d) A strong learning effect is observed between sessions. Reaction times decrease significantly from the first to the third session, regardless of the order TMS sessions.

similarity of the effects observed in the response timing and behavioural accuracy rules out speed-accuracy trade-off as an explanation for the observed effects.

Secondly, a 3 by 4 Repeated Measures GLM analysis was carried out to also assess the effects of TMS stimulation site on behavioural performance. The significant main effect of difficulty was preserved across TMS conditions ($F(3, 9) = 24.8$, $p < 0.001$). However, no main effect of TMS was observed ($F(2, 10) = 1.1$, $P = 0.38$) (Figure 4c), neither was there a significant interaction between difficulty and TMS ($F(6, 6) = 1.1$, $p = 0.48$). There was, on the other hand, a very strong effect of session order. As additional analyses revealed, regardless of TMS condition order which was randomised across participants, participants displayed a very consistent and very strong task learning effect between sessions. Entering session order into a Repeated Measures GLM procedure together with difficulty level revealed, besides the preserved difficulty effects, a significant main effect of session order ($F(2, 10) = 4.6$, $p = 0.039$), as well as a significant linear contrast ($F(1, 11) = 8.8$, $p = 0.013$) and a significant pairwise comparison between session 1 and 3 ($p = 0.038$) (Figure 4d). Between subsequent sessions, response time decreased on average with 587 and 235 ms respectively, and within single participants this could be as much as 2 seconds, thereby easily outweighing any potential increase in response time caused by TMS in the same session, which would be much more subtle. Although several single participants did show individual effects of TMS, the interpretation of such effects is difficult, since they are inherently flawed by the interwoven session learning effect. Separate

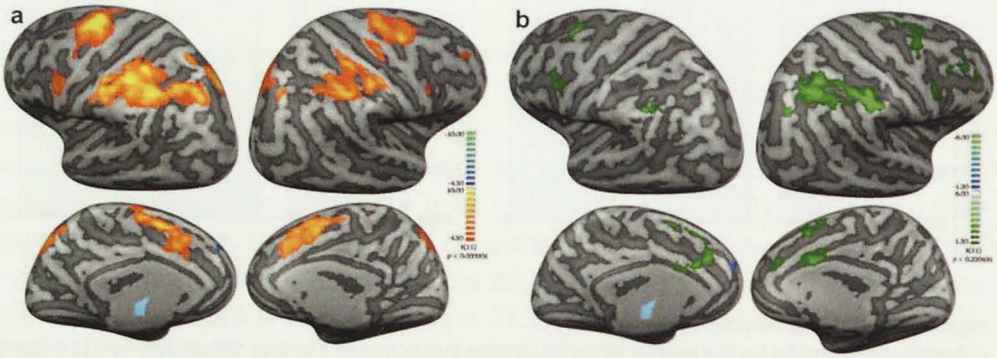

Figure 5. Random effects cortical activation maps

All depicted maps represent random effects analyses. Statistical activation maps projected onto cortex-based aligned average inflated hemispheres. A. Activation maps reflecting main activation as a result of the mental clock task. A bilateral network is shown consisting of posterior parietal (PPC), superior frontal, supplementary motor area (SMA) and anterior cingulate (ACC) cortical regions. B. Activation maps reflecting a conjunction analysis between main task effects (A) and parametrically modelled maps. Areas shown in green are brain regions displaying parametrically varying BOLD-activation in response to four levels of difficulty during the angle comparison phase of the task. This network is more lateralised to the right hemisphere, especially to right PPC.

classification of TMS and task learning effects was not possible, because in each participant, one session order was directly associated with one order of TMS.

fMRI results

Random effects staitical activation maps reflecting main activation as a result of the mental clock task disclosed a bilateral network, consisting of PPC, superior frontal, supplementary motor area (SMA) and anterior cingulate (ACC) cortical regions (Figure 5a). In addition, conjunction maps (main effects ∧ parametric effects) were produced depicting the parametric modelling of difficulty level (Figure 5b), which is supposedly reflecting the angle comparison phase of the mental clock task. These maps revealed brain regions displaying parametrically varying BOLD-activation in response to four levels of difficulty during the angle comparison phase of the task. This network is more lateralised to the right hemisphere, especially to right PPC, and is especially represented along the right intraparietal sulcus.

The effects of application of TMS to the left posterior parietal cortex were visualised using a simple random effects contrast of activation resulting from left PPC TMS, minus no TMS baseline (Figure 6a). Left PPC TMS resulted in increased BOLD in bilateral PPC, but especially in left superior and middle fontal cortex, and supplementary motor area (SMA). Areas appearing in blue are not likely to represent TMS deactivated regions, since no clear BOLD signal is observed in these regions. They are more likely to represent artefactual activation, probably resulting from non-overlapping functional slices obtained with partial brain scanning. Similarly, effects of application of TMS to the right posterior parietal cortex were visualised using a simple random effects contrast of activation resulting from right PPC TMS, minus no TMS baseline (Figure 6b). After right PPC TMS no notable changes in cortical activation were observed.

In addition, the effects of TMS on brain areas displaying high parametrical modulation were evaluated by contrasting parametric conjunction activation maps (main effects ∧ parametric effects) obtained after left or right TMS with that obtained after no TMS. After left PPC TMS, increased parametrically varying BOLD was observed in bilateral PPC, as well as in left superior and middle frontal sulci, all of which are more pronounced in the left hemisphere (Figure 6c). After right parietal TMS no such effects were observed (Figure 6d).

Discussion

After a brain lesion has occurred, a subset of patients experience partial functional recovery of initially lost abilities, especially during the first weeks and months after lesion onset. Exposing the neuronal mechanisms promoting such spontaneous

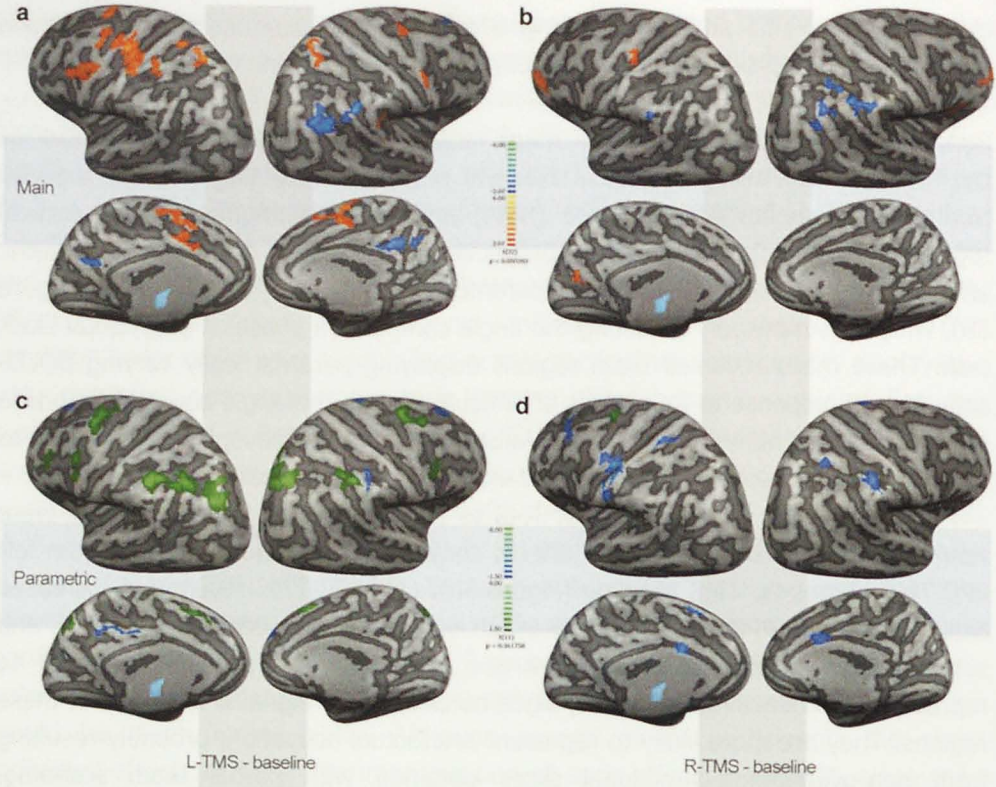

Figure 6. TMS effects on cortical activation patterns

All depicted maps represent random effects analyses. A. Contrast between left posterior parietal cortex (PPC) TMS stimulation compared to no TMS baseline. Left PPC TMS resulted in increased BOLD in bilateral PPC, but especially in left superior and middle fontal cortex, and supplementary motor area (SMA). Areas appearing in blue are not TMS deactivated regions, but represent artefactual activation, probably resulting from partial brain scanning. B. Random effects contrast between right posterior parietal cortex (PPC) TMS stimulation compared to no TMS baseline. After right PPC TMS no notable changes in cortical activation were observed. C. Conjunction between parametric and main activation maps created after left PPC TMS, contrasted to the same conjunction in no TMS baseline condition. Increased parametrically varying BOLD is observed in bilateral PPC, as well as in left superior and middle frontal sulci, all of which are more pronounced in the left hemisphere. D. Conjunction between parametric and main activation maps created after right PPC TMS, contrasted to the same conjunction in no TMS baseline condition. No notable differences in BOLD were observed.

recoveries, and which factors determine the extent of spontaneous recuperation in individual patients, is paramount in order to eventually design effective treatment. To date, the mechanisms underlying compensatory plastic processes are still elusive. In the current study magnetic brain stimulation and subsequent functional imaging were combined to visualise the effects of TMS on behavioural performance and brain activation during spatial mental imagery, and more specifically, to assess how preserved behavioural performance might be reflecting compensational brain activation patterns.

We employed the well-established mental clock task (MCT), one of few mental imagery tasks with behavioural output allowing to control for inaccuracy or lack of engagement regarding task execution. We expanded this paradigm with one newly added feature: a parametric modulation of the difference between the angles representing the two clocks. We hypothesised that this difference would not yet play any role during the mental imagery phase of task execution, since the interrelation between the two times is irrelevant for the formation of the two separate mental images. In contrast, during the angle comparison phase, a smaller difference between the two angles was hypothesised to result in increased task difficulty, which would subsequently be reflected in increased response times. The behavioural results corroborated this hypothesis, showing significantly increased response times with smaller angle differences, as well as a significant linear contrast. This observation warrants further treatment of this modulation as a manipulation of task difficulty. A very similar modulation was observed at the level of task accuracy, which both confirms the effective manipulation of task difficulty, and rules out speed-accuracy trade-off accounts as an alternative explanation for the observed effects.

In addition to the expected effects at the behavioural level, we hypothesised that BOLD-signals recorded from brain areas predominantly occupied with the angle comparison phase of the MCT would show similar modulations, allowing for a top-down dissociation between brain areas involved in mental imagery formation, and brain areas involved in spatial angle comparison. Main effects of the MCT revealed a network consisting of bilateral parietal and frontal areas, as expected. Using parametric modelling analysis, a subset of these brain regions was exposed, displaying BOLD-modulation in response to task difficulty, and hence, supposedly reflecting the spatial angle comparison phase of the MCT. These regions consisted of bilateral frontal and parietal areas, but especially right intraparietal sulcus. Thus, although a bilateral frontoparietal network is involved in MCT execution, a part of this network is especially reflecting spatial angle comparison processes, and this process is mostly confined to right PPC. This observation was in line with our expectations and evidence from previous studies.

Having established that the difficulty modulation allowing for top-down dissociation between mental imagery and spatial angle comparison was effective, we assessed the effects of virtual lesions to left versus right PPC on brain and behaviour. We employed the recently developed patterned continuous theta burst protocol (cTBS) (Huang et al., 2005). Whilst taking only 40 seconds of magnetic stimulation at a relatively low intensity, cTBS has been shown to reduce cortical excitation for a duration outlasting the actual stimulation period of approximately one hour. This makes cTBS into an attractive and effective protocol to allow for

post-hoc functional imaging of the cortical effects of the applied stimulation. We applied cTBS over individually, functionally determined left or right PPC 'hotspots', and started fMRI acquisition immediately afterwards, thereby visualising the effects of these lesions both on behavioural performance and on cortical activation patterns. Based on previous results we hypothesised that a TMS induced virtual lesion to the left PPC might evoke instantaneous functional reorganisation of brain activity, thus preserving behavioural performance during spatial mental imagery, whereas this reorganisation would not be observed after right PPC disruption, resulting in behavioural impairment.

As expected, no behavioural impairment was observed after left PPC disruption, possibly indicating instantaneous functional compensation by additional brain regions. Unfortunately however, the expected behavioural impairment following right PPC disruption was not observed. Considering the considerable behavioural improvement between sequential sessions, most likely caused by a massive extrasession learning effect, it is very likely that any TMS effect on behavioural performance – which would be of a much smaller order of magnitude – was masked. After all, significant behavioural impairments after right, but not left, PPC has been observed in several previous studies employing the exact same paradigm (Sack et al., 2005; Sack et al., 2002). Since previous studies employing the MCT either carried out a single experimental session per participant (Formisano et al., 2002; Sack et al., 2005; Trojano et al., 2000), or used a design allowing for intrasession comparison of TMS effects to baseline (Sack et al., 2005; Sack et al., 2002), there was no prior knowledge on an extrasession learning effect of this magnitude. The application of TMS was fully balanced across the 12 participants, yet at an individual level there was nevertheless a large influence of session order, in some cases as much as 2 seconds increase in response time, wiping out potential TMS effects taking place in the same session. Balancing session order might be an effective countermeasure with very large participant samples, but in costly studies of the current scale this is not a feasible option. Training each participant until the task is fully overlearned would be another option, but considering that the learning effect carried on into the third session – and possibly even further – this would be a disproportionally time consuming effort. However, a possible solution might be to design the experiment such that it allows for intrasession comparison between TMS and baseline conditions. In practice, this would inevitably require concurrent application of TMS and fMRI – hence, applying virtual lesions or otherwise, while simultaneously imaging task-related brain activation, and recording behavioural responses. As discussed in the introductory chapter of this thesis, simultaneous application of TMS and fMRI is indeed feasible, and might provide interesting insights beyond the simple fact that it controls for a

learning effect. In effect, our group is currently proceeding with this logical and promising next step.

Although no behavioural effects of TMS were observed, this does not automatically dismiss the effectivity of TMS per se. By definition, response times only show a single outcome measure, resulting from several cascading and parallel processes, which can only be assessed separately by systematically varying task characteristics. In this case, separation of these processes – i.e., effects of TMS regardless of effects of extrasession learning – was flawed, meaning that although TMS effects were masked in the behavioural outcome measure, TMS might still have affected behavioural and cortical processes. Consequently, we observed statistical activation maps contrasting TMS application to baseline. After left PPC disruption, an increase in BOLD was observed in bilateral superior parietal cortices, but especially in left middle frontal and precentral regions. Interestingly, such an increase was not observed after right PPC TMS. The increased cortical activation after left PPC TMS might very well reflect an instantaneous cortical reorganisation, aiming at preserving behaviour despite the presence of a virtual lesion. Interestingly, this reorganisation recruited not so much the right PPC, as was hypothesised, as ipsilateral frontal regions. Hence, failure of left parietal cortex might be compensated for by increased employment of frontal cortices in the ipsilateral hemisphere.

As previously described, the manipulation of difficulty level resulted in modulation at the behavioural as well as the cortical level, allowing for dissociation between mental imagery and spatial comparison processes at both levels. Parametric modelling of the BOLD showed that the angle comparison process remained largely confined to right intraparietal sulcus. Consequently, left versus right TMS might differentially affect the ability of the brain to deal with more or less heavy task demands. After left PPC TMS, an increase in parametrically modulated BOLD was observed across most of the functional network which was originally involved in MCT processing, but not previously displaying such a predisposition towards dealing with the angle comparison process. In contrast, after right PPC TMS, no changes in parametrically varying brain activation were observed. Bilateral frontal and parietal cortices were recruited after left PPC disruption, but most strongly displaying TMS modulation was left PPC itself. Since the exact sites of TMS stimulation were individually defined and thus anatomically slightly different, and the cortex based alignment procedure further clouds judgment about the exact position of individual stimulation sites, it is extremely likely that any local effects from applying TMS would get lost during group averaging. Hence, it is most probable that the increased parametric activation in left PPC represents an average of left PPC areas surrounding individual TMS

stimulation sites. Again, it seems that specifically after left PPC disruption, instantaneous functional reorganisation is set in motion.

In summary, the most likely explanation for the observed increases in general as well as parametric BOLD after left PPC disruption specifically is that, in response to this virtual lesion, the brain immediately starts re-routing processes to other brain areas in the same functional network, in an effort to maintain behavioural performance. This is a highly novel and fascinating observation. Interestingly, in contrast to expectations based on previous studies, it seems that mostly ipsilateral brain regions are increasingly engaged during these instantaneous reorganisations. Whether the lack of cortical activation changes after right PPC TMS reflects the hypothesised failure to deal with the virtual lesion – although in any case not catastrophic enough to survive the strong masking influence of the extrasession learning effect – or whether there is in effect no significant influence of TMS at all, is unfortunately impossibly stated with certainty. However, the latter would seem very unlikely, considering the previously found effects in single sessions using the same paradigm. Also, at an individual level, in some participants a decrease in response time is observed after right PPC TMS, whereas the session order would predict a decrease in response time due to learning. However, these observations are merely anecdotal.

In the current study, we specifically aimed to visualise the neuronal consequences of virtual brain lesions, and to reveal first direct insights into the neuronal mechanisms underlying compensatory brain processes supposedly arising to bypass a virtual lesion in order to maintain behavioural performance. We induced virtual lesions to left or right posterior parietal cortex using continuous theta burst TMS, a patterned protocol with inhibitive effects lasting for one hour after stimulation. Making use of this advantage to visualise the effects of TMS on both brain activation and behaviour, we started acquiring high quality functional imaging data immediately afterwards using event-related fMRI, while simultaneously recording behavioural data. Using a new, parametrically varied version of the mental clock task, we were able to for the first time top-down dissociate between mental imagery and subsequent spatial angle comparison stages of the task. Our results show that whereas mental imagery recruits frontoparietal networks bilaterally, angle comparison processing remains largely confined to the right intraparietal sulcus. Subsequently, by contrasting fMRI data acquired after cortical disruption with long-lasting TMS, we showed that specifically after left parietal TMS instantaneous compensational processes are set in motion, recruiting bilateral parietal but, surprisingly, most strongly ipsilateral frontal cortex. In addition, a large increase in compensational activation related to the spatial angle comparison phase of task processing was observed throughout the

functional network initially already involved in the mental clock task, but most strongly in left parietal regions surrounding the site of the virtual lesion. After right parietal TMS no such compensational effects on brain activation were observed. However, due to an unexpectedly strong learning effect masking potential TMS effects on behavioural performance, is it currently not clear whether this lack of activation indeed reflects a failure to compensate for the virtual lesion, or whether it reflects a general lack of effect of TMS at the behavioural level. Future studies, some of which are as of yet already in progress in the current group, should aim to simultaneously combine TMS disruption with functional imaging, so as to allow for intrasession comparison of TMS effects with baseline. Eventually, we hope that the results produced by the current and future studies lead to establishment of a neurobiological model of the dynamics and the asymmetry of compensatory processes, which might subsequently be applied to aid patient rehabilitation.

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Summary and conclusions

The general aim of the work described in the previous chapters was to explore how functional brain networks involved in higher cognition can be revealed, probed and perturbed using different combinations of research methods and analysis approaches. Conceptually, most of the chapters explored how different aspects of spatial cognition, such as visuospatial attention, multisensory spatial localisation, and size comparison of mentally imaged angles, are subserved by networks functionally linked to posterior parietal cortex. From a methodological viewpoint, a recurrent theme in this thesis is how the ever developing methods of brain imaging and brain interference might be combined to enable a unified perspective of functional brain networks responsible for the execution of complex, higher cognitive functions.

In *Chapter 2*, the neurodynamics underlying the previously supposed inhibition of automatic imitation were for the first time substantiated. The involved networks were visualised in healthy volunteers using conventional fMRI visualisation of statistical cortical activation maps, as well as Granger causality mapping, a form of effective connectivity analysis which reveals how different brain areas in the network causally influence each others task-related blood oxygen level dependent (BOLD)-activation. While these results were highly informative with regard to the brain regions involved in the task, they did not reveal which influence each brain area has on behavioural performance, more specifically on intentional imitation, automatic imitation and the inhibition of automatic imitation. Based on information provided by fMRI contrasts as well as by effective connectivity analysis, the integrity of the revealed network could be manipulated during task execution, using individually functionally neuronavigated TMS. Thus, the role of three important brain areas in the network could be specified and incorporated into an informed neurobiological model, and a neural correlate for the inhibition of automatic imitation was put forward. From a methodological angle, this chapter shows how different complementary methods and analysis approaches, in this case psychophysics, fMRI, effective connectivity analysis, and TMS brain interference, can be combined such that they complement each other, thereby exploiting the benefits of each approach, and compensating – if possible - for their respective limitations.

The aim of *Chapter 3* was to further investigate the known hemispheric asymmetry regarding spatial cognition, and probe whether right parietal cortex is indeed more vulnerable to spatial deficits, as suggested by lesion studies. By disrupting intraparietal cortex using TMS, a virtual form of contralesional extinction could be induced in healthy volunteers. Surprisingly, the "symptoms" of these virtual lesions were expressed after both left and right parietal TMS. Subsequently,

covert spatial attention was manipulated using an exogeneous cue, and the effects of this manipulation on the previously observed extinction symptoms were assessed. Depending on the hemifield of presentation –ipsilesional or contralesional- the manipulation was shown to enhance or, more importantly, counteract the TMS-induced behavioural deficits. In addition, a hemispheric asymmetry with regard to this interaction between attentional cueing and TMS was observed : an attentional cue appearing ipsilesionally impaired behaviour stronger after a virtual lesion to the right parietal cortex, than to the left. These observations speak to a model proposing an incapacity with regard to the redistribution of attention in the presence of an attention-binding distractor as a possible catalyst underlying the deficits characteristic of extinction. On a broader level, these results show how TMS might not only be used to evaluate which behavioural impairments occur after disruption of a certain brain area, but that it might also be employable as a tool for probing and evaluating rehabilitive treatment options in an early stage. This allows for random selection of 'patients', and for within-person balanced comparison between behaviour with and without 'treatment'. Such an approach increases explorative possibilities, limits the burden placed on the small amount of available acute lesion patients, and drastically reduces the impact of the many confounding factors inevitably associated with studying patients.

Chapter 4 deals with the influence of multisensory interactions and synesthetic associations on auditory spatial localisation. The hypothesis is put forward that widespread, subtle forms of synesthesia provide crossmodal mapping patterns which underlie and influence multisensory perception, especially in situations in which bottom-up integration cues are ambiguous. By combining psychophysics and ERP recordings, it was shown that despite systematic violations of spatial correspondence, the brain specifically integrates certain stimulus combinations which are congruent with respect to the hypothesis of pitch-size synesthesia, thereby impairing performance on an auditory spatial localisation task (spatial Ventriloquist illusion). Subsequently, this process was perturbed by functional disruption of the right intraparietal sulcus using TMS, with the result that the Ventriloquist effect was abolished, thereby increasing behavioural performance. By correlating between individual behavioural, TMS and ERP results, evidence was provided for shifting the current viewpoint on synesthesia more towards synesthesia being at the extremity of a spectrum of normal, adaptive perceptual processes, entailing close interplay between the different sensory systems. The results presented in this chapter support this spectrum view of synesthesia by demonstrating that its neural basis crucially depends on normal multisensory processes mediated by right posterior parietal cortex. From a more general

perspective, these results show how intact functioning of a certain brain area might result in behavioural impairment, and consequently, how disrupting such an area improves behavioural performance. This notion is particularly interesting in light of mental and affective conditions in which hyperactivity of certain brain areas results in impairment, in which case brain interference might be used to restore brain activations and hence behaviour to normal. In effect, TMS is already being applied in such a manner in clinical studies and even clinical treatment.

Chapter 5 zoomed in on yet a different aspect of spatial cognition, spatial mental imagery, with the explicit aim to visualise cortical compensational mechanisms supposedly taking place after a virtual lesion is inflicted upon posterior parietal cortex. Although brain plasticity occurring after a brain is lesioned can contribute greatly to patient rehabilitation, the mechanisms underlying spontaneous recovery are still elusive. Long-lasting TMS over left or right posterior parietal cortex was combined with subsequent fMRI, during which a spatial mental imagery task was executed. A right-hemisperic lateralisation was revealed, specifically in relation to the spatial component of mental imagery. In addition, it was shown that specifically after left parietal TMS instantaneous compensational processes are set in motion, surprisingly most strongly recruiting ipsilateral frontal cortex. The results presented in this chapter substantiate for the first time how the brain can instantaneously reroute functional activations in order to maintain behavioural performance. Future studies should aim at expanding this knowledge, and working up to improving these processes.

Taken together, several conclusions and insights arise from the results presented in this thesis. From a conceptual point of view, the observation that different aspects of spatial cognition causally rely on an intact posterior parietal cortex further substantiates the diverse functional contributions of parietal cortex to many complex cognitive processes. Also it seems that, rather than representing a single module responsible for a certain function, posterior parietal cortex possesses the ability to function as a mediator and modulator of complex cognitive functions processed in distributed networks of remote cortical areas. In addition, the observed hemispheric asymmetry with regard to parietal involvement further supports accounts of hemispheric specialisation or, like so, right hemispheric dominance with regard to spatial processing, a fact which directly impacts daily reality in many patients. The first visualisation of instantaneous functional reorganisation within a cortical network after disruption of parietal processing warrants and promotes further investigations into how and when the brain is able to compensate for functional disruptions, and how this process might be stimulated to improve patient rehabilitation.

From a methodological viewpoint, it has become increasingly clear that in order to investigate higher cognitive functions, it is often appropriate and in many cases even necessary to combine different available methods of cognitive neuroscience in order to be able to paint the whole picture. Although so far underrepresented in comparison to widely employed imaging techniques such as fMRI and EEG, brain interference seems to be at the heart of this, having won its spurs in probing functional network connectivity, among other applications. The work presented in this thesis shows how TMS can reveal neural network dynamics by locally and remotely affecting network functionality, and how disruption of brain regions identified with effective connectivity analysis can influence behavioural performance. It also demonstrates how TMS might not only be used to mimic neuropsychological defects, but additionally to evaluate treatments thereof. Furthermore, it reveals how instantaneous compensational processes may actually interact with such TMS-induced disruptions, making it appear from a behavioural viewpoint as if TMS had no effect at all, thus warranting visualisation of the effects of TMS on brain activation in addition to behaviour. In this sense, TMS may not only reveal the functional role of a specific brain area, but, more importantly, also how the rest of the brain copes with the local insult in terms of spontaneous functional reorganisation, compensation, and brain plasticity. As the latter observation already points out, great advances are to be expected from the simultaneous applications of imaging and interference techniques.

Several conceptual and methodological advances are thus presented in this thesis, but what are the implications of such advances? Cognitive neuroscience is by nature at the intersection of many disciplines. While it relies heavily on fundamental sciences like physics, cell biology and neuroscience, it largely leans towards psychology and cognitive sciences, and provides insights and even treatments used in neuropsychology, psychiatry and clinical practice. As science progresses and questions about the relationship between brain and behaviour are being solved, the more intricate questions remain. These questions require increasingly complex designs and analyses, and increasingly elaborate technical advances. As methods become more complex to answer relevant questions about the brain, a scientist has to become more and more of a specialist in using them. The risk carried by this paradox is an arising division between, on the one hand, methods specialists elaborating on more powerful but also more complex methods and analyses, and on the other hand researchers and clinicians which are hesitant to use these methods due to a lack of experience. In this light it is important to strive for a balance between methodological progression and usability, for example by creating user friendly software, providing training, and especially stimulating

cooperation and exchange of information between different disciplines. In the near future, fundamental and patient research will continue to approach the same goal from different directions: to unravel the functional architecture of the healthy brain, and use this knowledge to improve quality of life in millions of patients suffering from disorders of the brain.

Finally, just one view on how the future of cognitive neuroscience might look. Maybe thirty or fourty years from today, Joe Average gets up in the morning, and after taking his daily shower, he takes a seat under the households "Brain Balancer" – which might look somewhat like a hairdryer hood found at the average hair salon. He initializes the machine by a simple button press, telling it to start assessing the functional state of his brain. Within a minute or so, it informs him via a screen message about the current state of his brain. His left dorsolateral prefrontal cortex looks a bit overactive today, which just might affect his mood negatively – especially since his genetic background predisposes him towards developing a depressive state of mind. Consequently, the machine offers to settle this imbalance immediately; just press yes to proceed. Of course, Joe wants to start out his day with an evenly balanced brain, why not? Subsequently, the Brain Balancer uses its inbuilt Neuronavigator to automatically position an array of several magnetic coils, which are invisibly integrated into the hood, such that inhibitory magnetic stimulation is delivered exactly over Joe's left dorsolateral prefrontal cortex. After a minute of treatment, Joe feels refreshed, and confident that his brain can deal with anything he will encounter during this average working day. If the day turns out to be extra stressful, tonight he might use the Brain Balancer again to bring his overactive brain to relax more easily, who knows. That is, after his wife Judy has finished her anti-insomnia treatment for the night.

Although this scenario is far from realistic at the moment, and it is certainly questionable if it might ever reach that qualification, several advances in current cognitive neuroscience might point in this direction, resulting in this somewhat stretched extrapolation. For example, the benefits of more portable brain imaging setups such as functional near infrared spectroscopy (fNIRS) are already being explored. Analysis and classification software are steadily becoming more automatised and self-managing. With regard to brain stimulation, TMS is currently already being used as a valuable tool in treating otherwise treatment-resistant depressed patients, by inhibiting hyperactive areas and/or exciting hypoactive dorsolateral prefrontal areas which have been identified as catalysts in depression disorder. This treatment, offered throughout the USA by private Neurostar companies, has been approved by the American Food and Drug Administration (FDA). Moreover, a hand-held TMS device has already been developed for home treatment of migraine symptoms, advocated as a clinically proven, effective

alternative for use of medication, without treatment side-effects. This SpringTMS™ Total Migraine System delivers single TMS pulses according to settings saved on a prescription SIM card, and can be operated by the patients themselves, without the need of any additional assistance, whenever they want to use it. Additionally, there are advances such as the Brainsway Deep TMS system, which enables direct non-invasive stimulation of deep brain structures by employing an array of magnetic coils whose relative positions produce additive and cancelling magnetic fields, or the ANT-Neuro robotised TMS neuronavigation arm, which independently navigates a TMS-coil over a specified brain location, and compensates for (moderate) participant head motion by moving the coil along. Considering that cognitive neuroscience, its methods and its applications are continuously advancing, it is not entirely unthinkable that a futuristic scenario like the one described above might someday become daily reality. Whether we like it or not.

Samenvatting en conclusies

In het werk beschreven in de voorafgaande hoofdstukken werd onderzocht hoe functionele netwerken van hersengebieden aan het licht gebracht, onderzocht en gemanipuleerd kunnen worden, door de toepassing van een combinatie van verschillende onderzoeks- en analysemethoden. Inhoudelijk lag de nadruk in het merendeel van de hoofdstukken daarbij op het achterhalen hoe verschillende aspecten van ruimtelijke cognitie, zoals visuospatiële aandacht, multisensorische ruimtelijke localisatie en het vergelijken van de grootte van mentaal voorgestelde hoeken, gefaciliteerd worden door hersennetwerken die functioneel verbonden zijn met de posterieure pariëtaalcortex. Op methodologisch vlak loopt als een rode draad door dit proefschrift hoe de zich immer ontwikkelende methoden om de activatie van het brein weer te geven en te beïnvloeden gecombineerd kunnen worden, om uiteindelijk te leiden tot een coherente representatie van hoe functionele hersennetwerken de uitvoering van complexe, hoge cognitieve functies mogelijk maken.

In *Hoofdstuk 2* werden voor het eerst de neurodynamische processen verduidelijkt die ten grondslag liggen aan de inhibitie van automatische imitatie. Deze netwerken werden onthuld in gezonde vrijwilligers, niet alleen door het visualiseren van statistische corticale activatiepatronen met behulp van conventionele functionele kernspintomografie (fMRI), maar ook door het gebruik van Granger effectieve-connectiviteitsanalyse (GCM), een methode die aan het licht brengt hoe verschillende hersengebieden binnen een netwerk elkaar causaal beïnvloeden. Hoewel deze resultaten zeer informatief waren met betrekking tot welke gebieden bij de uitvoering van de taak betrokken waren, gaven ze geen inzicht in de manier waarop elk hersengebied precies gerelateerd was aan het kunnen uitvoeren van bepaalde functies, om precies te zijn intentionele imitatie, automatische imitatie en de inhibitie van automatische imitatie. Op basis van informatie afkomstig van zowel fMRI contrasten en effectieve-connectiviteitsanalyses kon het functioneren van het blootgelegde netwerk systematisch gemanipuleerd worden, met gebruikmaking van individueel neuro-genavigeerde transcraniale magnetische stimulatie (TMS). Op deze wijze kon de rol van drie belangrijke hersengebieden in het netwerk specifieker bepaald worden, en vervolgens kon deze informatie worden samengevoegd tot een eerste neurobiologisch model van de inhibitie van automatische imitatie. Vanuit methodologisch perspectief laat dit hoofdstuk zien hoe verschillende methoden en analyses, meer specifiek psychophysica, fMRI, effectieve-connectiviteitsanalyse en verstoring van hersenfuncties door TMS, gecombineerd kunnen worden op een manier dat ze elkaar complementeren, zodat de sterke kanten van elke methode optimaal benut kunnen worden, en de zwakke kanten voor zover mogelijk gecompenseerd.

Het onderzoek beschreven in *Hoofdstuk 3* had tot doel de hemisferische asymmetrie met betrekking tot spatiële aandacht nader te bekijken, en te verifiëren of de rechter pariëtaalcortex inderdaad kwetsbaarder is voor spatiële functieverstoringen, zoals lesiestudies doen vermoeden. Door de intrapariëtale cortex te verstoren met behulp van TMS, werd een virtuele vorm van contralesionale visuele extinctie geïnduceerd in gezonde vrijwilligers. Verrassend genoeg werden de "symptomen" van deze virtuele lesies zowel door TMS over de linker als over de rechter intrapariëtale cortex opgewekt. Vervolgens werd spatiële aandacht gemanipuleerd, en werden de effecten van deze manipulatie op de voorheen geobserveerde extinctie vastgesteld. Afhankelijk van in welk visueel veld de aandachtsmanipulatie gepresenteerd werd – ipsilesionaal of contralesionaal – bleek deze de verstorende effecten van TMS te kunnen versterken of, belangrijker nog, verminderen. Bovendien werd er een hemisferische asymmetrie geobserveerd met betrekking tot deze interactie: een ipsilesionaal gepresenteerde aandachtstrekker had een sterker negatief effect op de visuele waarneming wanneer een virtuele lesie was aangebracht in de rechter pariëtaalcortex, dan in de linker. Deze observaties steunen een model dat de lateralisatie vaak geobserveerd met betrekking tot extinctie in verband brengt met een asymmetrie in het vermogen om aandacht los te maken van een afleidende stimulus. In een breder perspectief laten deze resultaten zien dat TMS niet alleen gebruikt kan worden om te evalueren welke functies verstoord worden nadat een bepaald hersengebied verstoord werd, maar dat het wellicht eveneens inzetbaar is als een manier om rehabilitatieve behandelingen in een vroeg stadium van ontwikkeling uit te testen met gebruikmaking van gezonde vrijwilligers. Dit maakt een willekeurige selectie van 'patiënten' mogelijk, en een binnen-proefpersoonvergelijking tussen gedrag met en zonder 'behandeling'. Een dergelijke aanpak vergroot de exploratieve reikwijdte, reduceert de belasting op de kleine populatie beschikbare patiënten met acute lesies en verkleint de invloed van de vele verstorende factoren onvermijdelijk geassocieerd met patiëntenonderzoek drastisch.

Hoofdstuk 4 bespreekt de invloed van multisensorische interacties en synesthetische associaties op auditieve ruimtelijke localisatie. Synesthesie is een verschijnsel waarbij het waarnemen van een bepaalde stimulus tevens consistent waarneming van een andere stimulus veroorzaakt. De hypothese wordt gepresenteerd dat wijdverbreide, subtiele vormen van synesthesie zorgen voor verbindingspatronen tussen zintuigen, die de alledaagse multisensorische waarneming ondersteunen en beïnvloeden, in het bijzonder wanneer basale unisensorische stimuluskarakteristieken tegenstrijdige informatie verschaffen. Door psychofysica te combineren met taakgerelateerde hersenpotentialen (ERP) werd aangetoond dat het brein, in weerwil van een ruimtelijke discrepantie tussen de

visuele en auditieve signalen, specifieke combinaties hiervan integreert wanneer deze congruent zijn in het licht van de hypothese van toonhoogte-afmetingssynesthesie. Hierdoor wordt de prestatie op een auditieve ruimtelijke localisatietaak negatief beïnvloed (de ruimtelijke Buiksprekersillusie). Vervolgens werd dit integratieproces - en dus ook de Buiksprekersillusie - verstoord door stimulatie van de rechter intrapariëtale sulcus, een gebied belangrijk voor multisensorische integratie, met als gevolg dat de prestatie op de localisatietaak verbeterde. Door de correlatie vast te stellen tussen individuele prestaties op de taak, variaties in ERP potentialen, en het effect van TMS op beide, werd er nieuw bewijs geleverd voor een relatief recent standpunt dat synesthesie voorstelt als het ene extreme uiteinde van een spectrum van normale, adaptieve perceptuele processen; een proces dat vergezeld gaat van een intensieve wisselwerking tussen de verschillende zintuigen. De resultaten die in dit hoofdstuk gepresenteerd werden ondersteunen deze spectrumtheorie van synesthesie, door te demonstreren dat normale multisensorische processen die plaatsvinden in de rechter posterieure pariëtaalcortex van cruciaal belang zijn voor mild-synesthetische integratie. In een breder perspectief geplaatst laten deze resultaten zien hoe het ongehinderd functioneren van een bepaald hersengebied kan leiden tot slechter presteren op een bepaalde taak, en derhalve hoe het verstoren van de functie van dit hersengebied de prestatie kan verbeteren. Deze waarneming is met name interessant in het licht van bepaalde aandoeningen waarin hyperactiviteit van bepaalde hersengebieden een hoofdrol speelt, in geval waarvan magnetische verstoring van de hersenfunctie van nut zou kunnen zijn om de balans en het gedrag te herstellen. Inderdaad wordt TMS sinds kort op een dergelijke manier toegepast bij het onderzoeken en zelfs behandelen van bepaalde klinische aandoeningen.

Hoofdstuk 5 zoomt in op weer een iets ander aspect van ruimtelijke cognitie, namelijk het ruimtelijke mentale voorstellingsvermogen, met de expliciete doelstelling om compensationele processen te visualiseren die in de hersenen zouden plaatsvinden nadat een virtuele lesie wordt toegebracht aan de posterieure pariëtaalcortex. Hoewel reorganisatie van hersenfuncties na het optreden van een lesie van groot belang is voor de rehabilitatie van de patiënt, is er nog zeer weinig bekend over de mechanismen die ten grondslag liggen aan zulk spontaan herstel. Langwerkende TMS werd toegediend aan de linker of rechter posterieure pariëtaalcortex, waarna het effect van deze verstoring op het ruimtelijke voorstellingsvermogen werd vastgelegd met behulp van fMRI. Een rechterhemisferische lateralisatie werd onthuld, met name in relatie tot het ruimtelijke aspect van het mentale voorstellingsvermogen. Bovendien werd voor het eerst aangetoond dat specifiek na linkspariëtale verstoring compensationele

processen in werking treden, die hiervoor verrassend genoeg met name de linker frontaalcortex recruteren. De resultaten beschreven in dit hoofdstuk maken voor het eerst aanschouwelijk hoe het brein na een verstoring onmiddellijk functionele activaties kan omleiden om zo de uitvoering van functies zoveel mogelijk te handhaven. Toekomstige studies zouden deze processen verder moeten onderzoeken, en moeten toewerken naar een optimalisatie van deze spontane herstelprocessen.

Het werk beschreven in dit proefschrift leidt tot verschillende conclusies. Conceptueel gezien onderbouwt de observatie dat verschillende aspecten van ruimtelijke cognitie steunen op een intact functionerende posterieure pariëtaalcortex de assumptie dat dit hersengedeelte cruciaal is voor een scala aan complexe cognitieve processen. Daarbij lijkt de pariëtaalcortex niet zozeer een opzichzelfstaande module te zijn, verantwoordelijk voor een specifieke functie, maar eerder een mediator en regulator van complexe cognitieve processen die gefaciliteerd worden door netwerken van samenwerkende hersengebieden. Daarnaast staaft de geobserveerde hemisferische asymmetrie met betrekking tot de functies uitgevoerd door de pariëtaalcortex bestaande theoriëen met betrekking tot hemisferische specialisatie, om precies te zijn rechterhemisferische dominantie in de uitvoering van ruimtelijke processen, een feit dat de alledaagse realiteit van vele patiënten bepaalt. De eerste visualisatie van directe functionele reorganisatie binnen een corticaal netwerk nadat pariëtale functies verstoord werden rechtvaardigt verdere studies naar wanneer en hoe het brein in staat is functionele verstoringen te compenseren en hoe deze processen gestimuleerd kunnen worden om zo de rehabilitatie van patiënten te kunnen bevorderen.

Vanuit een methodologisch oogpunt wordt het steeds duidelijker dat om hogere cognitieve functies effectief in kaart te kunnen brengen, het combineren van de verschillende beschikbare methoden van de cognitieve neurowetenschappen vaak aangewezen, en in vele gevallen zelfs nodig is. Hoewel tot dusver ondergerepresenteerd in vergelijking met veelgebruikte beeldvormingstechnieken zoals fMRI en EEG, lijkt hersenstimulatie de spil te zijn in dit proces, met zijn vermogen om onder andere de connectiviteit binnen functionele netwerken bloot te leggen. Het werk beschreven in dit proefschrift laat zien hoe TMS de dynamische processen binnen een neuraal netwerk kan onthullen door zowel lokaal als elders in het netwerk de activiteit te veranderen, en hoe de verstoring van hersengebieden geïdentificeerd met connectiviteitsanalyses gedrag en prestatie kan beïnvloeden. Het laat ook zien hoe TMS niet alleen gebruikt kan worden om neuropsychologische defecten te imiteren, maar bovendien om behandelingen daarvan te evalueren. Daarnaast laat het zien hoe directe

compensatoire processen met TMS-geïnduceerde verstoringen kunnen interageren, waardoor het gedrag ongewijzigd blijft, en de schijn gewekt wordt dat TMS geen verstorend effect had. Dit brengt de noodzaak aan het licht om de effecten van TMS op hersenactivatie te kunnen visualiseren, een proces waar in dit proefschrift een begin mee is gemaakt. In lijn met deze laatste observatie is er grote vooruitgang te verwachten van de simultane toepassing van beeldvormings- en verstoringstechnieken.

In dit proefschrift worden dus verschillende conceptuele, alsook methodologische ontwikkelingen beschreven, maar wat zijn nu de implicaties van zulke ontwikkelingen? De cognitieve neurowetenschappen bewegen zich bij uitstek in het snijvlak van vele disciplines. Hoewel het zwaar steunt op fundamentele wetenschapsgebieden zoals fysica, celbiologie en neurowetenschappen, leunt het tevens richting de psychologie en de cognitieve wetenschappen, en worden er inzichten en standpunten aan ontleend die van belang zijn voor neuropsychologie, psychiatrie en de klinische behandelpraktijk. Terwijl de wetenschap voortschrijdt en meer en meer vragen over de samenhang tussen brein en gedrag beantwoord worden, blijven de meer complexe en intrigerende vraagstukken onbeantwoord. Om deze vraagstukken aan de orde te stellen zijn steeds complexere onderzoeksopzetten en analyses nodig, en steeds verder ontwikkelde technische vaardigheden. Naarmate de methoden nodig om relevante vragen met betrekking tot het brein te beantwoorden complexer worden, moet de wetenschapper meer en meer gespecialiseerd worden in het gebruik ervan. Het risico dat deze paradox met zich meebrengt is dat aan de ene kant methodologisch ontwikkelaars steeds krachtigere, maar ook steeds complexere technieken voortbrengen, maar dat aan de andere kant onderzoekers en behandelaars terughoudend zijn deze methoden toe te passen vanwege een gebrek aan ervaring. In het licht hiervan wordt des te meer duidelijk dat er gestreefd moet worden naar een balans tussen methodologische vooruitgang en bruikbaarheid, bijvoorbeeld door gebruiksvriendelijke software, het aanbieden van training, en in het bijzonder door interdisciplinaire samenwerking en de uitwisseling van informatie te bevorderen. In de nabije toekomst zullen fundamenteel onderzoek en patiëntenstudies hetzelfde doel trachten te naderen vanuit verschillende richtingen: het ontrafelen van de functionele architectuur van het gezonde brein, en het toepassen van deze kennis om de kwaliteit van leven te verbeteren van miljoenen patiënten die leiden aan hersenaandoeningen.

Tot slot, zomaar een mogelijk toekomstscenario van de cognitieve neurowetenschappen. Over een jaar of dertig, veertig staat Jan Jansen 's morgens op, en nadat hij zijn dagelijkse douche heeft genomen neemt hij plaats onder de

"Brain Balancer" van het gezin – een apparaat dat wel wat lijkt op een grote haardroger zoals die aangetroffen kan worden bij de kapsalon. Hij start het apparaat op met een simpele druk op de knop, en vraagt het de functionele toestand van zijn hersenen te vast te stellen. Na ongeveer een minuut is het proces voltooid, en op het display verschijnt een bericht dat hem informeert over de huidige toestand van zijn brein. Zijn linker dorsolaterale prefrontale cortex lijkt een beetje overactief vandaag, wat zijn humeur wellicht negatief zou kunnen beïnvloeden – vooral gezien zijn genetische achtergrond, die hem toch al kwetsbaarder maakt voor het ontwikkelen van een depressieve toestand. Gelukkig biedt het apparaat de mogelijkheid om deze toestand van onbalans direct op te lossen; gewoon op ja drukken om door te gaan. Natuurlijk wil Jan de dag graag beginnen met een uitgebalanceerd brein, waarom niet? Vervolgens gebruikt de Brain Balancer zijn ingebouwde Neuronavigator om een reeks van magnetische spoelen, die onzichtbaar geïntegreerd zijn in de kap, zodanig te positioneren dat inhiberende magnetische stimulatie precies toegediend wordt aan Jan's linker dorsolaterale prefrontale cortex. Na een minuutje van behandeling voelt Jan zich helemaal opgefrist, en hij is er zeker van dat zijn brein alles aankan waar het mee te maken krijgt op een gemiddelde werkdag. Wanneer zijn werkdag extra stressvol blijkt te worden, zal hij vanavond wellicht de Brain Balancer gebruiken om zijn overactieve brein tot rust te brengen. Tenminste, nadat zijn vrouw Julie haar slapeloosheidsbehandeling voor de nacht heeft afgerond.

Hoewel dit scenario op dit moment nog verre van realiteit is, en het zeker de vraag is of het dat ooit zal worden, zijn er verschillende ontwikkelingen gaande in de cognitieve neurowetenschappen die de deur openen naar dergelijke scenario's, waardoor dit ietwat vergezochte toekomstbeeld niet helemaal onvoorstelbaar is. Zo wordt er actief gezocht naar meer mobiele beeldvormingstechnieken, waarbij functionele bijna-infrarood spectroscopie (fNIRS) veelbelovend lijkt. Software voor analyse en classificatie van gegevens wordt steeds meer automatisch en zelfsturend. Met betrekking tot hersenstimulatie is er ook continu vooruitgang te bespeuren. Momenteel wordt TMS reeds toegepast als waardevolle behandelingsmethode van depressieve patiënten die niet of nauwelijks meer reageren op conventionele behandeling, waarbij overactieve hersengebieden die reeds geïdentificeerd zijn als stoorzenders in depressieve aandoeningen onderdrukt worden, of juist onderactieve gebieden geactiveerd. Deze behandeling, die in de Verenigde Staten aangeboden wordt door private Neurostar ondernemingen, is reeds erkend door de Amerikaanse nationale Voedsel- en Medicijnenautoriteit (FDA). Daarnaast is er voor zelfstandige thuisbehandeling van migrainesymptomen recentelijk een handheld TMS-apparaat ontwikkeld, dat aangeprezen wordt als een klinisch bewezen en effectief alternatief

voor medicinale behandeling, zonder de gevreesde bijwerkingen daarvan. Dit SpringTMS™ Total Migraine System dient een enkele TMS puls toe aan de hand van specificaties opgeslagen in een SIM-kaart, die enkel op recept verkrijgbaar is, en het kan gebruikt worden door de patiënt zelf, zonder verdere assistentie, wanneer de patiënt dit nodig acht. Daarnaast zijn er ontwikkelingen zoals het Brainsway Deep TMS System, dat naast de cortex ook dieper gelegen subcorticale hersenstructuren weet te stimuleren door het combineren van de magnetische velden van een reeks van magnetische spoelen. Of de ANT Neuro robotarm, die zelfstandig een TMS spoel precies boven een gespecificeerde locatie in de hersenen navigeert, en die meebeweegt met een licht bewegende proefpersoon. De snelheid waarmee de cognitieve neurowetenschappen, en haar methoden en toepassingen zich ontwikkelen in aanmerking genomen, is het niet geheel ondenkbaar dat een futuristisch scenario zoals dat hiervoor geschetst op een dag werkelijkheid kan worden. Of we dat willen of niet.

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Finally thanks to Alexander Supertramp, for some inspiring new views on the world, and reminding me once more about the things in life that really matter.

Now I hope I didn't forget anyone, but just in case... thanks to you, too!

Curriculum Vitae

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Nina Bien was born the 14th of November 1980 in Heerlen, The Netherlands. In 1999 she completed her secondary school education at St. Maartenscollege in Maastricht. From 1999 until 2001 she studied Cinematography at the Narafi institute in Brussels, Belgium. In 2001 she started her studies in Biological Psychology at Maastricht University, during which she worked part time as a camera operator at the provincial TV station L1. After following a specialisation in Cognitive Neuroscience, and completing a research internship with Dr. Alard Roebroeck and Dr. Alexander Sack, she obtained her Master's degree with distinction in 2006. She continued the fMRI and TMS research initiated during her internship as a research assistant with Dr. Alexander T. Sack, a position funded by the 'Future Talent' initiative of Maastricht University. In 2007 she started working as a PhD Candidate at the department of Cognitive Neuroscience, under the supervision of Dr. Alexander Sack and Prof. Rainer Goebel. After obtaining her PhD degree in 2011, she will continue her work as a postdoctoral fellow.

Published articles and abstracts

Bien, N., Goebel, R., and Sack, A.T. (2010). Combining TMS and fMRI to visualise cortical compensational mechanisms during mental imagery. Poster presented at the annual meeting of Society for Neuroscience, San Diego, USA.

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